From INSTITUTE OF ENVIRONMENTAL MEDICINE
Karolinska Institutet, Stockholm, Sweden

DIETARY CARBOHYDRATE QUALITY AND HEALTH – FOCUS ON LOW-GRADE SYSTEMIC INFLAMMATION AND CARDIOMETABOLIC RISK FACTORS

Nor Adila Mhd Omar

Karolinska Institutet

Stockholm 2020

Published by Karolinska Institutet.
Printed by Universitetsservice US-AB, 2020

ISBN 978-91-7831-963-3
Cover illustration by Yazid Shariff (behance.net/yxji)

Dietary carbohydrate quality and health – focus on low-grade systemic inflammation and cardiometabolic risk factors
THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Nor Adila Mhd Omar

Principal Supervisor:
Professor Rikard Landberg
Chalmers University of Technology
Dept. of Biology and Biological Engineering
Division of Food and Nutrition Science

Co-supervisor(s):
Professor Alicja Wolk
Karolinska Institutet
Institute of Environmental Medicine
Unit of Cardiovascular and Nutrition Epidemiology

Associate Professor Galia Zamaratskaia
Swedish University of Agricultural Sciences
Department of Molecular Sciences

Opponent:
Associate Professor Emily Sonestedt
Lund University
Department of Clinical Sciences Malmö
Division of Nutritional Epidemiology

Examination Board:
Professor Johan Ärnlöv
Karolinska Institutet
Department of Neurobiology

Professor Margareta Nyman
Lund University
Department of Food Technology, Engineering and Nutrition

Associate Professor Helen Mary Lindqvist
SU Sahlgrenska
Department of Internal Medicine and Clinical Nutrition

Dedicated to my beloved parents and lovely family

"We always want to move forward, and to move forward,
we need to develop new knowledge"

Mahathir Mohamad

ABSTRACT

Dietary carbohydrate quality, characterized by content of whole grain (WG), dietary fiber, and sugars, is important for human health. Whole grain consumption and cereal fiber can reduce the risk of chronic disorders such as cardiovascular disease (CVD) and type 2 diabetes, an effect partly mediated by alterations in cardiometabolic risk factors and low-grade systemic inflammation. High-fructose or high-galactose diets may trigger pro-inflammatory and negative metabolic effects, but fermentable dietary fiber, *e.g*., fructooligosaccharides (FOS), may mitigate these effects. This thesis examined the effect of dietary carbohydrate quality, characterized by intake of specific WGs and sugars, on low-grade systemic inflammation and cardiometabolic risk factors. In particular, it evaluated: 1) associations between long-term WG rye or wheat intake and inflammatory, endothelial function, and CVD risk-related biomarkers; 2) alkylresorcinols (AR) in adipose tissue as potential biomarkers of long-term WG intake; 3) effects of WG/bran rye and refined wheat on inflammatory and endothelial function biomarkers in individuals with low-grade prostate cancer; and effects of high fructose and galactose intake, with or without added FOS, on 4) selected metabolic factors and inflammatory and gut permeability biomarkers; and 5) modulation of gut microbiota in rats.

Data and samples from two prospective cohort studies, Swedish Mammography Cohort-Clinical (SMC-C) (n=109) and Cohort of Swedish Men-Clinical (COSM-C) (n=149), were used to analyze associations between long-term WG intake and selected biomarkers of inflammation, endothelial function, and metabolic factors. WG intake was assessed by food frequency questionnaires and using alkylresorcinols (AR) in plasma and adipose tissue as biomarkers. Combined WG rye and WG wheat intake was positively associated with cathepsin S, while total AR in plasma was inversely associated with endostatin (adjusted for age, sex, and BMI). Long-term WG rye intake was modestly correlated (r=0.31-0.41) with AR in adipose tissue, whereas WG wheat was poorly correlated (r=0.17-0.33) over 14 years for men and 17 years for women. The effect of WG/bran rye on selected inflammatory biomarkers was explored in a dietary intervention cross-over study of 17 men with low-grade prostate cancer. TNF-R2, e-selectin, and endostatin were significantly lower in these men after consumption of WG/bran rye products than consumption of refined wheat with added cellulose. In an animal study with rats (n=6/group and time point) fed a high-fructose or high-galactose diet, with/without added FOS, or three control diets for six or 12 weeks, the intervention diets affected several metabolic factors and gut integrity markers, but not inflammation biomarkers. High-fructose and high-galactose diets did not cause substantial changes in gut microbiota composition, but addition of FOS favored the genus *Bifidobacterium*. Gut microbiota was associated with several metabolic and inflammation biomarkers.

These results suggest that WG wheat and rye may have positive impacts on some inflammation biomarkers. Fructose and particularly galactose had adverse metabolic effects in rats, but no obvious effect on inflammation markers. Sugars did not markedly affect gut microbiota composition in rats.

ABSTRAKT

Kostens kolhydratkvalitet som kan beskrivasutifrån innehållet av fullkorn, kostfiber och sockerarter, har en viktig inverkan på människors hälsa. Ett högt intag av fullkornintag och spannmålsfibrer visat sig leda till minskad risk att utveckla kroniska sjukdomar, såsom hjärt- och kärlsjukdomar och typ 2-diabetes. Riskminskningen medieras delvis genom förändringar i kardiometaboliska riskfaktorer och låggradig systemisk inflammation. En kost med högt innehåll av fruktos eller galaktos kan utlösa proinflammatoriska och negativa metaboliska effekter. Fermenterbara kostfibrer, såsom fruktooligosackarider (FOS), har föreslagits mildra sådana effekter. Det övergripande syftet med denna avhandling var att undersöka hur kostens kolhydratkvalitet, med avseenede påfullkorn från råg och vete samt sockerarter, påverkar kardiometaboliska riskfaktorer och låggradig systemisk inflammation.

De specifika syftena var: 1) analysera sambandet mellan långvarigt intag av fullkornsråg och vete och biomarkörer för inflammation, endotelfunktion och kardivaskulärsjukdom; 2) utvärdera alkylresorsinoler (AR) i fettvävnad som potentiella biomarkörer för långvarigt fullkornsintag; 3) analysera effekterna av fullkorn/rågkli och vittvete på biomarkörer för inflammation/endotelfunktion hos individer med låggradig prostatacancer; 4) utvärdera effekterna av högt fruktos- och galaktosintag med eller utan tillsatt FOS på utvalda metaboliska faktorer samt biomarköer för inflammation och tarmpermeabilitet; 5) i en råttmodell utvärdera effekterna av högt fruktos- och galaktosintag med eller utan tillsatt FOS på tarmflorans sammansättning.

Data från två prospektiva kohortstudier, Swedish Mammography Cohort-Clinical (SMC-C) (n=109) och Cohort of Swedish Men-Clinical (COSM) (n=149), användes för att analysera sambandet mellan långvarigt fullkornsintag – fastställt via livsmedelsfrekvensformulär samt med AR som biomarkör i plasma och i fettvävnad. Även utvalda biomarkörer för inflammation, endotelfunktion och metaboliska faktorer inkluderades. Summan av fullkornsråg och-veteintag var positivt associerad med cathepsin S, och total AR i plasma var omvänt associerad med endostatin (justerat för ålder, kön och BMI) (**Paper I**). Samma data och prover användes för att utvärdera AR i fettvävnad som en biomarkör för långvarigt fullkornsintag. Långvarigt intag av fullkorn var måttligt korrelerat (r=0.31-0.41) med AR i fettvävnad, medan fullkornsvete hade en lägre korrelation (r=0.17-0.33) under 14 år för män och 17 år för kvinnor (**Paper II**). En crossover-studie på 17 män med låggradig prostatacancer användes för att undersöka effekten av fullkorn/ rågkli på utvalda inflammatoriska biomarkörer. TNF-R2, e-selectin och endostatin var signifikant lägre hos män efter konsumtion av fullkorn/ rågkliprodukter jämfört med vitt vete med tillsatt cellulosa (**Paper III**). I en djurstudie matades råttor (n=6/grupp och tidpunkt) antingen med hög fruktos- eller höggalaktosdiet med eller utan tillsatt FOS jämfört med tre kontrolldieter under 6 eller 12 veckor. Interventionsdieterna påverkade flera metabola faktorer och tarmintegritetsmarkörer, men inte inflammationsbiomarkörer (**Paper IV**). Kost med hög fruktos och hög galaktos orsakade inte betydande förändringar i tarmflorans sammansättning medan tillsats av FOS stimulerade Bifidobacterium-släktet. Tarmflorans

sammansättning var associerad med flera metaboliska och inflammatoriska biomarkörer (**Paper V**).

Sammanfattningsvis tyder resultaten på att fullkornsvete och fullkornsråg kan ha en positiv inverkan på vissa inflammationsbiomarkörer. Dessutom visade sig fruktos och särskilt galaktos ha negativa metaboliska effekter hos råttor, men ingen påtaglig effekt på inflammationsmarkörer. Sockerarter hade inga markanta effekter på tarmflorans sammansättning hos råtta. Randomiserade, kontrollerade studier på människor behöver genomföras för att bekräfta dessa resultat.

LIST OF SCIENTIFIC PAPERS IN THE THESIS

I. **Nor Adila Mhd Omar**, Huaxing Wu, Anders Larsson, Alicja Wolk, Rikard Landberg.
Long term whole grain rye and wheat consumption and their associations with selected biomarkers of inflammation, endothelial function and cardiovascular disease. *European Journal of Clinical Nutrition* 2020. DOI: 10.1038/s41430-020-00714-3

II. Huaxing Wu, **Nor Adila Mhd Omar**, Niclas Håkansson, Alicja Wolk, Karl Michaëlsson and Rikard Landberg.
Evaluation of alkylresorcinols in adipose tissue biopsies as a long-term biomarker of whole-grain wheat and rye intake in free-living Swedish men and women. *Public Health Nutrition* 2018; 21(10), 1933-1942.

III. Galia Zamaratskaia, **Nor Adila Mhd Omar**, Carl Brunius, Göran Hallmans, Jan-Erik Johansson, Sven-Olof Andersson, Anders Larsson, Per Åman, Rikard Landberg.
Consumption of whole grain/bran rye instead of refined wheat decrease concentrations of TNF-R2, e-selectin, and endostatin in an exploratory study in men with prostate cancer. *Clinical Nutrition.* 2019. DOI: https://doi.org/10.1016/j.clnu.2019.01.007

IV. **Nor Adila Mhd Omar**, Jan Frank, Johanita Kruger, Federica Dal Bello, Claudio Medana, Massimo Collino, Galia Zamaratskaia, Karl Michaëlsson, Alicja Wolk, Rikard Landberg.
Effects of high intakes of fructose and galactose with or without added fructooligosaccharides on metabolic factors, inflammation and gut integrity in a rat model. *Submitted.*

V. **Nor Adila Mhd Omar**, Johan Dicksved, Johanita Kruger, Galia Zamaratskaia, Karl Michaëlsson, Alicja Wolk, Jan Frank, Rikard Landberg.
Effect of a diet rich in galactose or fructose with or without fructooligosaccharides on gut microbiota consumption in rats. *Submitted.*

CONTENTS

1 Introduction 1
2 Background 3
2.1 Cardiometabolic diseases and risk factors 3
2.2 Low-grade systemic inflammation – a risk factor in cardiometabolic disease 6
2.3 Whole grains and cardiometabolic risk factors 8
2.3.1 Measurement of whole grain intake 8
2.3.2 Effects of different grains on inflammation 10
2.4 The role of gut fermentation and fiber-mediated effects on inflammation and cardiometabolic risk factors 11
2.5 The effects of common dietary sugars on cardiometabolic risk factors and inflammation 13
2.5.1 Effects of high fructose and high galactose intake on cardiometabolic risk factors and inflammation 14
3 Aims 17
4 Subjects and methods 19
4.1 Study designs 19
4.1.1 Swedish Mammography Cohort–Clinical and Cohort of Swedish Men–Clinical 20
4.1.2 Whole grain intake 21
4.1.3 Alkylresorcinols 21
4.1.4 A whole grain/rye bran intervention study in men with prostate cancer 22
4.1.5 Experimental study in rats 23
4.1.6 Fructose and galactose 24
4.1.7 Fructooligosaccharides (FOS) 24
4.2 Selected biomarkers analyzed as outcomes in the different studies 25
4.3 Gut microbiota 26
4.4 Statistical analyses 26
5 Results and discussion 29
5.1 Long-term whole grain intake, biomarkers of whole grain intake, and low-grade inflammation in observational studies 29
5.1.1 Baseline characteristics of men and women in Papers I and II 29
5.1.2 Associations between self-reported whole grain rye and wheat intake, alkylresorcinols as intake biomarkers, and inflammatory, endothelial function, and CVD-risk related markers (Paper I) 30
5.1.3 Alkylresorcinols in adipose tissue as biomarkers of long-term whole grain intake (Paper II) 32
5.2 Effects of whole grain/bran rye consumption on inflammatory, endothelial function, and CVD risk-related biomarkers 34

5.3 Effects of high fructose and high galactose intake, with or without added fructoologosaccharides, in rats 36
5.3.1 Energy intake and body weight 36
5.3.2 Effects on blood glucose and insulin 38
5.3.3 Effects on blood lipids 40
5.3.4 Effects on inflammatory biomarkers 42
5.3.5 Effects on advanced glycation end products 42
5.3.6 Effects on gut permeability markers 44
5.3.7 Effects on gut microbiota richness and diversity 46
5.3.8 Effects on relative abundance of gut microbiota composition 47
5.3.9 Association between gut microbiota and selected metabolic, inflammatory, and gut permeability markers 49
5.3.10 Methodological considerations 51
6 General discussion 53
7 Main findings and conclusions 55
8 Future research 56
9 Acknowledgements 57
10 References 59

LIST OF ABBREVIATIONS

AGEs	Advanced glycation end products
ALP	Alkaline phosphatase
ALT	Alanine transaminase
ANCOVA	Analysis of covariate
ANOVA	Analysis of variance
AR	Alkylresorcinol(s)
AST	Aspartate transaminase
BMI	Body mass index
BW	Body weight
CHD	Coronary heart disease
CKD	Chronic kidney disease
CMDs	Cardiometabolic diseases
CML	N_{ε}-carboxymethyl-lysine
CO_2	Carbon dioxide
COSM	Cohort of Swedish Men
CORR	Correlation
COSM-C	Cohort of Swedish Men-Clinical
CRP	C-reactive protein
CVD	Cardiovascular disease
DNA	Deoxyribonucleic acid
EGFr	Epidermal growth factor receptor
ELISA	Enzyme-linked immunosorbent assay
FFQ	Food frequency questionnaire
FOS	Fructooligosaccharides
GI	Glycemic index
GIP	Glucose-dependent insulinotropic peptide-1
GLM	Generalized linear model
GLP	Glucagon-like-peptide-1
HbA1c	Hemoglobin A1c
HDL	High-density lipoprotein
HOMA-IR	Homeostatic Model Assessment for Insulin Resistance
hsCRP	High-sensitivity C-reactive protein
IAP	Intestinal alkaline phosphatase
ICAM-1	Intercellular adhesion molecule-1
ICCI	Intra-class correlation
IDL	Intermediate-density lipoprotein
IFG	Impaired fasting glucose
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IGT	Impaired glucose tolerance
IL	Interleukins
IL-10	Interleukin-10
IL-13	Interleukin-13
IL-1Ra	Interleukin-1 receptor antagonist
IL-1α	Interleukin-1 alpha
IL-1β	Interleukin-1 beta
IL-4	Interleukin-4
IL-5	Interleukin-5
IL-6	Interleukin-6

IL-8	Interleukin-8
Kcal	Kilocalories
LDL	Low-density lipoprotein
LPS	Lipopolysaccharides
MHC	Major histocompatibility complex
n-6 PUFA	n-6 polyunsaturated fatty acids
NALFD	Non-alcoholic fatty liver disease
NCDs	Non-communicable diseases
NF-κB	Nuclear factor-kappa B
OTU	Operational taxonomic unit
P13K	Phosphatidylinitisol-3-kinase
PAI-1	Plasminogen activator inhibitor 1
PAR_2	Protease-activated receptor 2
PCA	Principal component analysis
PCR	Polymerase chain reaction
PKC	Protein kinase C
RCTs	Randomized controlled trials
RP	WG/bran rye products
ROS	Reactive oxygen species
rRNA	Ribosomal ribonucleic acid
SCFA	Short-chain fatty acids
SMC	Swedish Mammography Cohort
SMC-C	Swedish Mammography Cohort-Clinical
T2D	Type 2 diabetes
TNF	Tumor necrosis factor
TNF-R1	Tumor necrosis factor receptor 1
TNF-R2	Tumor necrosis factor receptor 2
TNF-α	Tumor necrosis factor-alpha
TNF-β	Tumor necrosis factor-beta
TWG	Total whole grains
VCAM-1	Vascular cell adhesion molecule-1
VEGF	Vascular endothelial growth factor
VLDL	Very low-density lipoprotein
WG	Whole grain
WGP	Whole grain product
WGR	Whole grain rye
WGR/WGRnW	Whole grain rye to whole grain rye and wheat intake ratio
WGRnW	Whole grain rye and wheat
WGW	Whole grain wheat
WHO	World Health Organization
WP	Refined wheat grain products

1 INTRODUCTION

Cardiometabolic diseases (CMDs) comprise different disorders, including cardiovascular disease (CVD), diabetes type 2 (T2D), and chronic kidney disease (CKD). CMDs evolve over a long time and risk factors such as insulin resistance, hyperglycemia, dyslipidemia, obesity, and hypertension, which are regarded as key components of metabolic syndrome, play a central role in the etiology [1, 2]. Low-grade systemic inflammation is regarded as a hallmark of pathological features of CMDs and has therefore been suggested as a key component in CMDs [3]. One strategy for prevention of CMDs is to alter modifiable risk factors. Diet is an important modifier of such risk factors, and a healthy diet, along with improved lifestyle habits, could reduce, reverse, and prevent CMDs [4, 5].

Carbohydrates represent a heterogeneous group of molecules consisting of carbon, hydrogen, and oxygen atoms, and are the main source of energy and dietary fiber in the human diet [6-8]. Amount and composition of the carbohydrates in the diet are therefore of great importance for health and wellbeing [3, 9].

Carbohydrate quality in the diet can be measured in various ways and can be described by characterization of amounts and types of whole grain (WG), dietary fiber, and their sources, and by glycemic index (GI) [10]. In this thesis, simple sugars were also included as a component of carbohydrate quality, due to their implied negative effects on health [8, 11].

In recent decades, there has been growing evidence that high WG intake is associated with reduced risk of several non-communicable diseases and their common risk factors [10, 12]. Epidemiological studies have reported an inverse association between high WG intake and the incidence of CVDs, T2D, CKD, and cancers [12]. Randomized control trials (RCTs) have shown beneficial effects of high WG intake on body weight, cholesterol level, and systolic blood pressure [13-15], but results for many outcomes are inconclusive [10, 16].

Whole grain foods are one of the main contributors to cereal fiber intake in the diet [17]. Meta-analyses have shown that high intake of cereal fiber decreases the risk of all-cause and CVD-specific mortality, and also the incidence of T2D, coronary heart disease (CHD), and colorectal cancer [10, 16, 18]. However, while reductions in disease risk appear to be consistent in observational epidemiological studies, results from RCTs on cardiometabolic risk factors appear to be less consistent [10, 16].

Simple sugars are readily consumed in the diet. Studies on both humans and animals have shown clear adverse effects of high intake of simple sugars on cardiometabolic conditions such as insulin resistance, diabetes, dyslipidemia, and obesity [19, 20]. High fructose and high galactose intake in animals has been shown to enhance expression of pro-inflammatory cytokines and induce activation of inflammatory cascades and endothelial function [21-24]. However, only a small number of studies have investigated the role of carbohydrate quality, including simple sugars, on inflammation, and the mechanisms underlying observed effects are

still poorly understood. Moreover, few studies have investigated the impact of sugars on the composition of gut microbiota. Glycemic index, a measure of glycemic response to carbohydrate-containing foods, is an important dimension of carbohydrate quality and is influenced by various factors, including types of sugars and fibers consumed and total energy intake [25, 26]. However, a recent meta-analysis showed that the associations between GI and cardiometabolic health outcomes are weaker and less consistent than for WG and cereal fiber [10]. Therefore, GI was not the main focus in this thesis.

Health effects of WG have mainly been attributed to dietary fiber content and composition, and associated phytochemicals, but also to food structure and indirect effects on gut microbiota [12, 27-29]. However, most studies on the effect of carbohydrate on gut microbiota have focused on dietary fiber, while very few have considered the effects of sugars [30, 31]. Moreover, many studies have investigated the effects of total WG intake on metabolic risk factors, without acknowledging that grains differ in amount and composition of dietary fiber and phytochemicals. Few studies have investigated the effects of specific grains, particularly minor grains such as rye, which is the richest source of dietary fiber among the cereals. In most observational studies, self-reported data have been used to evaluate the association between WG intake and metabolic factors, rather than an objective measure such as dietary biomarkers that could provide complementary information [32, 33].

2 BACKGROUND

2.1 CARDIOMETABOLIC DISEASES AND RISK FACTORS

Cardiometabolic diseases comprise a cluster of disorders that can cause dysregulation of the metabolic system in the body [34], leading to CVD, T2D, and CKD [35-37]. Important risk factors in these underlying diseases, referred to as cardiometabolic risk factors, include obesity, insulin resistance, hyperglycemia, dyslipidemia, hypertension, and vascular dysfunction.

There is growing evidence that low-grade inflammation plays a role in the pathophysiology of many chronic diseases, and low-grade systemic inflammation has been studied as a target for prevention and intervention in many studies [38, 39]. Low-grade systemic inflammation has been shown to be closely associated with cardiometabolic risk factors [40]. Elevation of C-reactive protein (CRP), a major acute protein, has been linked with metabolic dysfunction, particularly abnormal glucose metabolism through insulin resistance [41]. Proatherogenic and prothrombic features of CRP result in abnormal lipid metabolism [39]. Moreover, increased expression of CRP, tumor necrosis factor α (TNF-α), and interleukin-1 beta (IL-1β) has been observed in T2D and CVD patients, indicating the important role of low-grade inflammation in CMDs [38].

Reducing cardiometabolic risk factors is regarded as an important strategy to control CMDs [1]. Modern lifestyle features, including poor diet, smoking, lack of exercise, and high alcohol consumption, are the major modifiable risk factors in CMDs [35]. Changing dietary and lifestyle habits is an important prevention strategy to reduce the prevalence of CMDs through effects on cardiometabolic risk factors [42, 43].

Obesity

Overweight and obesity can be defined as excessive or abnormal accumulation of fat that may cause detrimental effects on health [44]. Body mass index (BMI, kg/m^2) is commonly used to determine overweight and obesity. For adults in Western countries, overweight is defined as BMI $\geq$25 kg/m^2, while obesity is defined as BMI $\geq$ 30 kg/m^2 [45]. The etiology of obesity is multifactorial, including an interaction between genetics, hormones, and environment [45]. Energy imbalance between calories consumed and expended, due to dietary and lifestyle habits, is the fundamental reason for overweight and obesity [46, 47].

Obesity is a major determinant of many of the other cardiometabolic risk factors [48, 49]. Obesity aggravates dyslipidemia, insulin resistance, and hypertension [50, 51]. Abdominal obesity is strongly correlated with insulin resistance, higher low-density lipoprotein (LDL), and lower high-density lipoprotein (HDL) [52]. Individuals with BMI >35 kg/m^2 have a higher risk of developing T2D than individuals with normal weight [37, 43]. Elevated LDL cholesterol may contribute to accelerated atherogenesis [53]. Moreover, the majority of obese individuals, but not all, display insulin resistance [54]. Studies in obese subjects with impaired glucose tolerance have shown a reduction in the incidence of new-onset diabetes after weight reduction achieved

by control of food intake combined with consistent physical activity [51, 55]. Obesity is preventable and could be avoided by reducing energy intake, increasing physical activity, and increasing consumption of whole grains, vegetables, and fruits [35, 56]. Reducing body weight is one of the strategies to improve health and lower cardiometabolic risk factors such as blood pressure, blood glucose, insulin sensitivity, triglycerides, and increased HDL cholesterol [57]. Type and amount of carbohydrates consumed have been shown to play a role in long-term weight management [58].

Hyperglycemia and insulin resistance

Hyperglycemia is defined as an abnormally high concentration of glucose in circulation in the body. Hyperglycemia occurs due to the inability of beta cells to produce the hormone insulin or to the inability of insulin to bind to protein receptors on the target cell membrane [59].

Hyperglycemia is a condition that indicates pre-diabetes or diabetes [59, 60]. Several important factors that cause hyperglycemia include large meals containing too much sugar, physical inactivity, and not taking anti-diabetic medication properly [61, 62]. Chronic hyperglycemia may result in accumulation of advanced glycation end products (AGEs) and subsequent impaired renal function, because the kidney is the major site of AGE clearance [63-65]. Accumulation of AGEs can lead to endothelial dysfunction, increased oxidized LDL in plasma, lower LDL uptake, and suppressed repair of the vascular system [63, 66]. High concentrations of AGEs in various tissues have also been linked with greater severity of atherosclerotic plaque and oxidative stress [63].

Insulin resistance or impaired glucose tolerance is a metabolic condition where the body does not respond to insulin in the liver or in the muscles [67]. Under normal conditions, insulin binds to the protein receptors found on the plasma membrane of target cells and causes the cells to take in more glucose molecules from the blood. However, under insulin resistance conditions, the cells do not respond to the insulin due to an impaired signaling pathway and the beta cells therefore compensate by generating more insulin. At some point, the production of insulin becomes exhausted, despite high demand, and T2D develops [67, 68].

Before manifestation of T2D, impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) will occur. IFG and IGT are pre-diabetic conditions that refer to the state of abnormal regulation of glucose between normal glucose homeostasis and diabetes [69]. Such states can be reversible with improved lifestyle, including improved diet [70]. Normal fasting blood glucose concentration is less than 5.6 mmol/L and returns to below 7.8 mmol/L by two hours after eating. Briefly, IFG is defined as an increase in concentration of fasting plasma glucose to ≥5.6 but <6.9 mmol/L, whereas IGT is defined as an increase in plasma glucose concentration to ≥ 7.8 but <11.1 mmol/L, after an oral glucose tolerance test with 75 g glucose load. Individuals with increased IFG and/or IGT tend to develop diabetes at different progression rates [69]. Apart

from T2D, other common conditions associated with insulin resistance are dyslipidemia, hypertension, and metabolic syndrome [67].

Dyslipidemia

Dyslipidemia is an abnormal lipid profile of one or several lipids or lipoproteins in circulation [71]. The lipid profile includes lipoproteins carrying cholesterol (HDL, LDL, very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL)) and triglycerides. Atherogenic dyslipidemia is characterized by imbalanced proatherogenic and antiatherogenic concentrations, such as LDL and HDL, respectively, in circulation [72]. HDL has atheroprotective properties, and low HDL has been used as a strong independent risk factor to predict CVD in healthy individuals [73].

High LDL and triglycerides and low HDL concentrations are main components of cardiometabolic diseases [74-76]. High LDL concentration is frequently associated with poor diet, obesity, kidney or liver disease, and certain types of medication [75, 77]. High LDL concentration increases the risk of CVDs, especially coronary heart disease (CHD), with the CVD risk decreasing with reductions in LDL concentration [78].

Diet and lifestyle interventions and selective medication are used in individuals with high cardiovascular risk, in order to reduce the risk. Statins were initially developed to treat hypercholesterolemia and have been linked with a significant reduction in cardiovascular events [79]. Moreover, most CHD patients have a low HDL concentration. Low HDL cholesterol can be a predictive risk factor for CHD and is only slightly improved by statin therapy [35, 80].

Hypertension

Hypertension is a common condition in which the force exerted on the walls of arteries by the blood consistently exceeds normal ranges. Globally, more than 1 billion people have been diagnosed with hypertension and this figure is projected to exceed 1.6 billion by 2025 [81]. Hypertension is rarely accompanied by symptoms until serious complications arise. Long-term and uncontrolled hypertension results in thickening and narrowing of the blood vessels and significantly raises the risk of myocardial infarction, heart failure, stroke, and CKD [82].

A strong correlation has been reported between hypertension and obesity, diabetes, dyslipidemia, and insulin resistance, *i.e.*, the core pathophysiological features of metabolic syndrome [35, 82, 83]. The majority of diabetes patients have hypertension, together with other cardiometabolic risk factors including dyslipidemia, abdominal adiposity, and low-grade systemic inflammation [84, 85]. In fact, the primary causes of hypertension are poor diet and lack of exercise [35, 86].

2.2 LOW-GRADE SYSTEMIC INFLAMMATION – A RISK FACTOR IN CARDIOMETABOLIC DISEASE

Low-grade systemic inflammation plays a critical role in the etiology of chronic diseases and often occurs without symptoms [40, 87]. During a state of low-grade systemic inflammation, tissues express high concentrations of immune cells and inflammatory factors. This does not cause any structural changes or loss of primary function, with levels remaining in the healthy range [40]. However, prolonged low-grade inflammation has been suggested as a causal risk factor for many chronic diseases such as CVD and diabetes [88-90]. During inflammation, immune cells produce inflammatory factors known as cytokines to signal other immune cells and coordinate complementary cascades to fight against 'non-self' molecules [40]. Inflammation status can be clinically evaluated by measuring the concentrations of cytokines, other inflammatory biomarkers, white blood cells, and fibrinogen in the blood [91].

Elevated expression of high-sensitivity C-reactive protein (hsCRP), an acute-phase inflammatory protein produced by the liver, is commonly used to reflect low-grade systemic inflammation [92, 93]. Expression of hsCRP can signal many conditions, including infections, CVD, cancers, and autoimmune diseases [91]. During inflammatory disorders, hsCRP binds to phospholipids of pathogens or damaged cells, and initiates a complementary system. Interleukin-6 (IL-6) is released by activation of T-cells and macrophages, and is the main inducer of hsCRP gene expression [92]. In human studies, circulating concentrations of inflammatory biomarkers such as hsCRP , IL-6, interleukin-10 (IL-10), and tumor necrosis factor α (TNF-α) have been associated with increased risk of developing T2D [94], obesity [95], and metabolic syndrome [96]. High expression of CRP and of TNF-α is associated with obesity and could mediate insulin resistance, which leads to manifestation of metabolic syndrome [90].

Cytokines are small proteins produced by cells (including immune cells such as macrophages, B and T lymphocytes, and mast cells) in response to pathogens and other antigens that regulate immune responses. Cytokines include interleukins, chemokines, tumor necrosis factor (TNF), interferons, and lymphokines [97, 98]. Interleukins can have both pro- and anti-inflammatory properties. The primary roles of interleukins are modulation of growth and activation and differentiation of immune cells during inflammation. Interleukins are also involved in proliferation, adhesion, maturation, and migration of immune cells. Interleukins consists of a large group of proteins that can evoke many reactions in cells and tissues and have some redundant functions [99]. For instance, interlukin-4 (IL-4), interleukin-5 (IL-5), and interleukin-13 (IL-13) are involved in stimulating B-cell differentiation and growth. IL-6 is produced by B and T lymphocytes and macrophages, and has primary effects on differentiation of B cells and stimulation of acute-phase protein. TNF is an important cytokine that contributes to both physiological and pathological processes, systematic inflammation, tumor lysis, apoptosis, and initiation of acute-phase reaction [100].

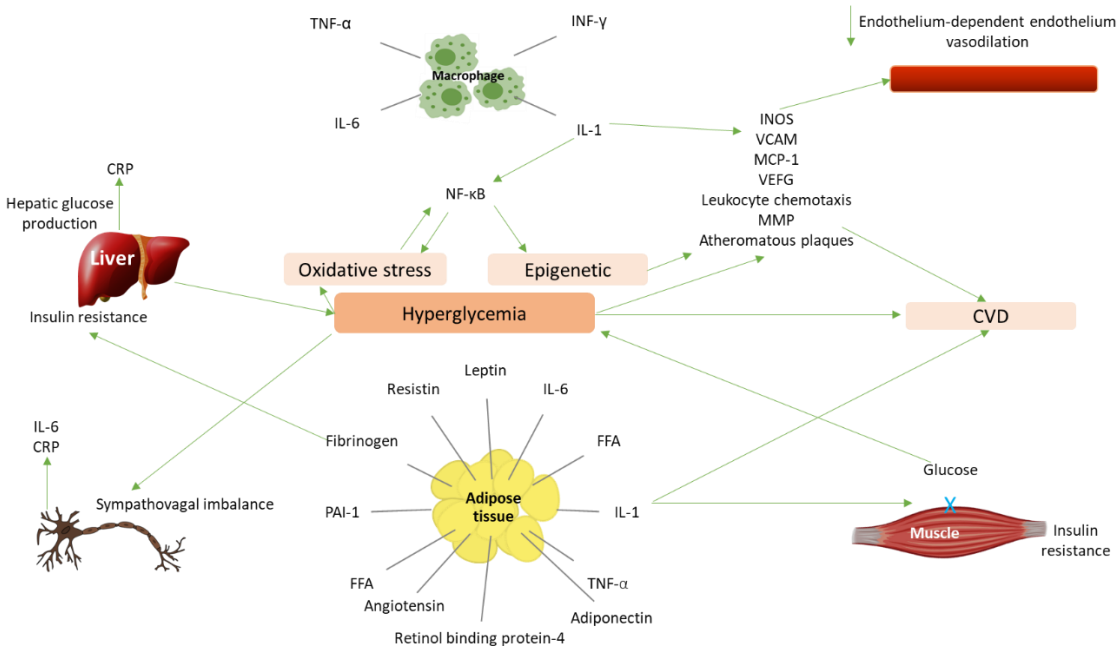

Figure 1. Schematic overview of the role of low-grade inflammation in diabetes and cardiovascular disease (Modified from Matheus *et al.* [64]).

The TNF family includes TNF-alpha (TNF-α), TNF-beta (TNF-β), and their ligands. All TNF family members are involved in activation of nuclear factor-κB (NF-κB) (101). As a key mediator of both acute and chronic systematic inflammation responses, TNF also stimulates other cytokines and chemokines [100]. Moreover, TNF interacts with its two receptors, TNF receptor 1 (TNF-R1) and TNF-R2, which belong to a subgroup of the TNF receptor superfamily [102]. TNF-R1 plays an important role in apoptosis, necroptosis, and activation of NF-κB, and is expressed by any type of cell [102, 103]. Unlike TNF-R1, TNF-R2 is not involved in cell death-inducing activity, but it also stimulates activation of NF-κB [102, 104]. Expression of TNF-R2 is limited to only a few types of cells, such as regulatory T-cells, myeloid cells, epithelial cells, and some tumor cells [104].

The interrelations between inflammation, obesity, and insulin resistance have been thoroughly investigated, but are complex. Expression of pro-inflammatory cytokines induces insulin resistance by inhibiting the signal transduction of insulin in skeletal muscles, liver, and adipose tissue [105, 106]. Higher CRP, IL-6, TNF-α, and interleukin-8 (IL-8) concentrations in circulation have been observed in insulin-resistant individuals [105, 107]. Moreover, in individuals with insulin resistance, higher glucose concentration in circulation results in higher uptake by endothelial cells [108]. Higher glucose inside the endothelial cells produces reactive oxygen species (ROS) as a by-product. Increased ROS level leads to formation of AGEs and stimulation of protein kinase C (PKC), which increases the production of vascular endothelial growth factor (VEGF) and other growth factors that result in angiogenesis and cell growth. PKC also increases production of endothelin, which can stimulate platelet aggregation. In addition, PKC is involved in increased NF-kB production. The net result from this activation

is increased expression of inflammatory receptors and increased vascular permeability through stimulation of cytokine production. Essentially, the increased vascular permeability and receptor expression allow monocytes circulating in the blood and LDL to pass through the endothelial layer and enter the *tunica intima*. As they enter the tissues, macrophages engulf LDL and become foam cells. They also release cytokines and chemokines, which causes further inflammation, produce more ROS, and convert LDL into oxidized LDL. This cycle is important in initiation and progression of atherosclerosis, a chronic inflammatory condition caused by a continuous supply of atherogenic oxidized LDL. Apoptosis and necrosis of foam cells contribute to formation of a lipid-rich core within the plaque. The foam cells also secrete growth factor, cytokines, and chemokines, causing smooth muscle proliferation and migration into the *tunica intima*. Smooth muscle cells secrete extracellular matrix substances such as collagen, and this fibroproliferative response stabilizes the plaque. *Tunica intima* thickening by fibroproliferative response and lipid accumulation results in atherosclerotic plaque formation. Disruption of atherosclerotic plaque leads to atherothrombosis [109]. In animal models with atherosclerosis, reductions in plasma lipids have been shown to profoundly affect production of plaque in arteries and to reduce many inflammatory processes [89]. Therefore, it has been suggested that lowering inflammatory markers could significantly reduce the incidence of various chronic diseases [93].

2.3 WHOLE GRAINS AND CARDIOMETABOLIC RISK FACTORS

Whole grain (WG) contains all three parts of the grain kernel (bran, germ, and the starchy endosperm) in similar proportions as in the intact grain [110]. The bran and germ are rich in dietary fiber, vitamins, and bioactive compounds such as polyphenols and phenolic acids, vitamin B, minerals, proteins, and fats. The starchy endosperm contains mainly starch, but also some proteins. Whole grains of rye, wheat, corn, brown rice, oats, sorghum, and barley differ in their composition and quality of bioactive compounds, dietary fiber, and other nutrients. When grain is milled into refined flour, most of the beneficial compounds from the other parts and from the germ are removed, leaving the starch-rich endosperm and some proteins [111].

2.3.1 Measurement of whole grain intake

A limitation in most observational studies investigating the role of diet on health is that they rely on self-reported dietary data, which may lead to relatively large measurement errors [112, 113]. In dietary intervention studies, lack of compliance is an issue which could also lead to problems when interpreting the results. One way to overcome these difficulties could be to introduce dietary biomarkers, to be used as independent measures of specific food intakes or compliance measurements in dietary interventions. Currently, only a few valid dietary biomarkers exist [114].

Alkylresorcinols (ARs) have been suggested and evaluated as biomarkers of whole grain wheat and rye intake [115-118]. Recently, ARs have been used as a tool to reflect whole grain intake from wheat and rye in epidemiological studies and in whole grain interventions [117]. ARs constitute a group of phenolic lipids (1,3-dihydroxy-5-alkylbenzene derivatives) mainly found in the outer layer of rye and wheat grains. The concentration of ARs varies in different whole grains, ranging from 720-761 µg/g for rye, 489-1429 µg/g for wheat, and 42-51 µg/g for barley, while ARs are not found in any appreciable amounts in oats, maize, millet, rice, and sorghum [117]. Alkylresorcinol homologs ranging in size from C17:0 to C25:0 form specific profiles in different cereals. The ratio of C17:0 to C21:0 homologs is 1.0 for rye and 0.1 for wheat, and has been used to determine the source of whole grain in food products [115, 117].

In humans, ARs are absorbed from the upper intestine via the lymphatic system, and can be quantitatively determined in human plasma, urine, feces, and adipose tissues [116, 119, 120]. The half-life of ARs is around five hours and they are completely eliminated from plasma after 140 hours [121]. Previous small-scale studies have suggested that AR concentrations in adipose tissue may serve as a long-term biomarker of whole grain wheat and rye intake [122, 123]. However, observational studies comparing plasma AR with adipose tissue AR concentrations are lacking in the literature.

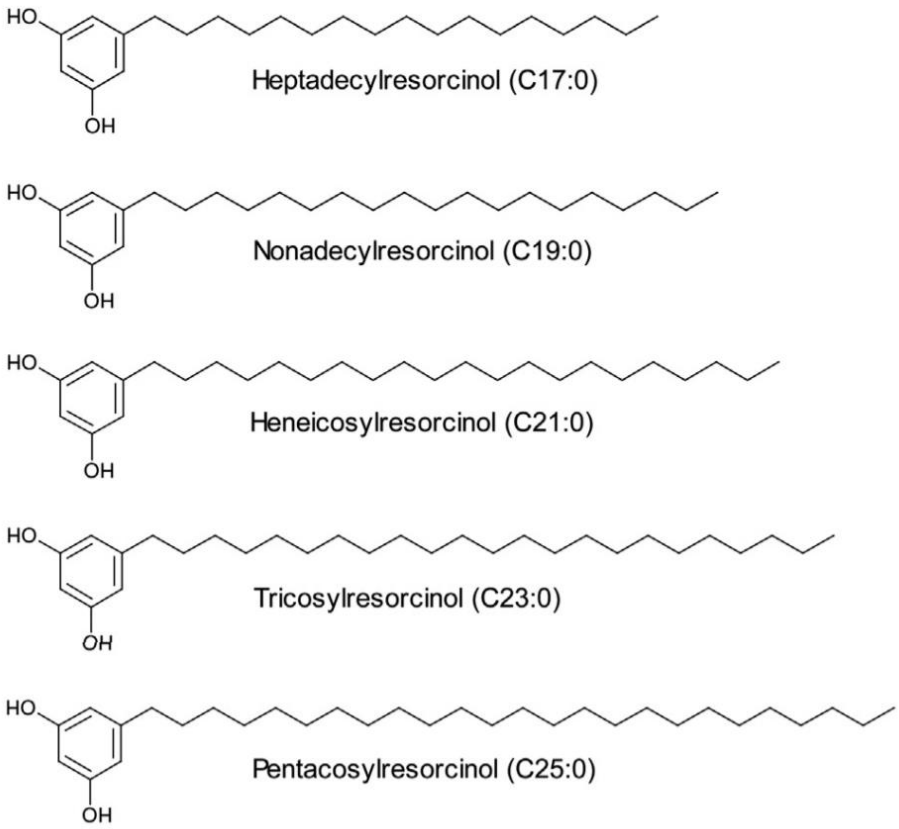

Figure 2. Structure of five major alkylresorcinol homologs (source: Ziegler *et al*. [124]).

2.3.2 Effects of different grains on inflammation

To date, observational studies and RCTs have reported mixed findings on the effects of whole grains on inflammation [125]. Whole grain intake is reported to be inversely associated with CRP and plasminogen activator inhibitor 1 (PAI-1) concentration [126]. Consumption of WG wheat has been shown to cause no alteration in hsCRP and IL-6 concentrations in healthy individuals [127]. In overweight and obese individuals, consumption of WG wheat has been found to cause a reduction in TNF-α concentration after eight weeks and increased anti-inflammatory cytokine concentration (IL-10) after four weeks, compared with refined wheat [128].

Consumption of WG rye did not change IL-6 concentration in healthy individuals in an overnight crossover study [129]. A reduction in IL-6 was observed in individuals at risk of developing metabolic syndrome after eight weeks of consumption of whole grains, particularly rye [130]. Rye and oat diets, compared with the typical Western diet, lower alkaline phosphatase (ALP) and alanine aminotransferase (ALT) concentrations, an effect associated with alleviation of TNF and toll-like receptor 4 [131]. Moreover, rye and barley intake lowers acute inflammatory responses and is correlated with glycemic response and gut fermentation [132-134]. In addition, an oat-enriched diet has been shown to improve inflammatory status compared with a control diet [135]. Four weeks of consumption of oat porridge has been found to significantly lower the concentrations of hsCRP, TNF-α, IL-6, and IL-8 in hypercholesterolemic individuals [136].

In animal studies, it has been shown that the small particle size of wheat bran improves the inflammatory status in fructose-induced metabolic syndrome in mice [137]. In a previous study with an obese rat model, rats treated with wheat bran had reduced PAI-1 concentration compared with rats treated with α-cellulose, but there were no effects on IL-6 or IL-1β concentrations [138]. Mice fed wheat bran had lowered TNF-α and IL-6 concentrations, and enhanced IL-10 in response to LPS-induced inflammation, compared with mice fed a control [139]. Treatment using ARs from various wheat brans on LPS-treated macrophage RAW 264.7 cells was found to result in lower expression of NF-κB and inhibitor κB kinase [140], suggesting that AR could be an active component of WG affecting inflammation.

Although the mechanisms behind the anti-inflammatory and protective effects of whole grains remain unclear, several promising pieces of evidence indicate that short-chain fatty acids (SCFAs), which are produced in the gut from WG fiber, may be a major contributor [141].

2.4 THE ROLE OF GUT FERMENTATION AND FIBER-MEDIATED EFFECTS ON INFLAMMATION AND CARDIOMETABOLIC RISK FACTORS

The interactive role of diet and gut microbiota composition and function on human health is currently a very active area of research. The human gut contains a complex and diverse community of microorganisms that colonize different parts of gastrointestinal tract, but mainly the large intestine [28]. The most abundant bacteria found in healthy individuals are anaerobes dominated by the phyla Bacteroidetes and Firmicutes [29, 142, 143]. Other phyla found at lower levels include Actinobacteria, Proteobacteria, and Verrucomicrobia [28, 29]. Gut microbiota plays an essential role in many aspects of human health and diseases, including maturation and stimulation of the immune system, modulation of metabolic activities, and protection of the host from gastrointestinal infection [144, 145].

Complex carbohydrates are important in shaping the whole microbiota community in the large intestine, through modulation of gut microbiota richness and diversity [146-148]. Greater microbial richness and diversity are associated with improved health status [149]. Dietary fiber is the main food component responsible for the increased richness and diversity of gut microbiota in general and of SCFA-producing bacteria such as *Faecalibacterium prausnitzii* and *Roseburia* spp. in particular. Fermentable carbohydrates such as fructooligosaccharides (FOS), inulin, and galactooligosaccharides (GOS) are fermented by gut microbiota, producing diverse beneficial substances such as SCFAs. Human and animal studies have demonstrated that FOS and inulin can promote the growth of *Bifidobacterium* species [150, 151]. Higher abundance of *Bifidobacterium* has been linked with a range of health benefits, including protective effects on gut barrier and stimulation of the immune system [151, 152]. Fructans are also reported to enhance the growth of *Faecalibacterium prauznitzii*, a butyrate-producing bacterial species [28, 150]. However, lower levels of supplementation with fructans may impair the growth of *Bacteroides* and *Clostridium* [28].

Alteration of gut microbiota composition has been associated with cardiometabolic risk factors and low-grade inflammation [153-155]. Dysbiosis or imbalance of microbiota composition increases pathobionts and decreases non-pathogenic bacteria. Dysbiosis has been implicated in gut barrier dysfunction and enhanced translocation into the circulation of lipopolysaccharides (LPS), a major component of the cell membrane in Gram-negative bacteria [155]. The immunogenic impact of LPS involves increased expression of pro-inflammatory biomarkers such as TNF-α, CRP, and IL-6 as a response of the immune system. In general, insulin resistance and dyslipidemia have been associated with low diversity of gut microbiota. Individuals with obesity, CVD, and T2D have been shown to host lower abundances of specific bacteria such as *Bifidobacteria* and *Akkermansia* species [156].

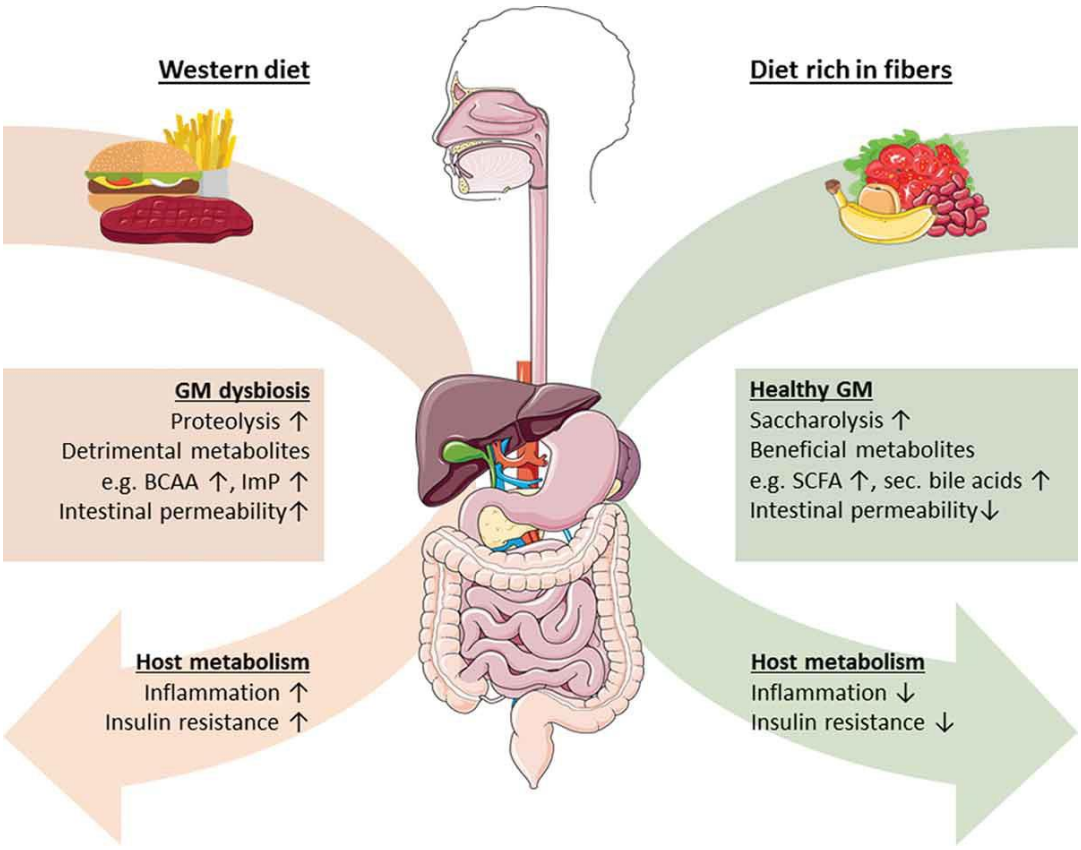

Figure 3. Overview of the effects of (left) the Western diet and (right) a diet rich in fiber on health of the gut microbiota (GM) and on host metabolism. (source: Warmbrunn *et al.* [153]).
BCAA – branched-chain amino acids, SCFA – short-chain fatty acids.

Moreover, microbiota-derived metabolites, mainly SCFAs, have been identified as playing a primary role in maintenance of health and development of diseases [157]. SCFAs are organic acids with 2-carbon to 5-carbon weak acids, including acetate (C2), propionate (C3), butyrate (C4), and valerate (C5), and primary products of non-digestible carbohydrate fermentation. Concentrations of acetate, propionate, and butyrate in the colonic lumen vary depending on the type of dietary intake [158-160]. A higher concentration of these fermentation products lowers the pH in the colon and increases butyrate formation [161].

Many studies have investigated the association between SCFAs and gut integrity and glucose homeostasis, and the effects of SCFAs on lipid metabolism, appetite regulation, and immune function [162]. SCFAs also affect metabolic health and CVD risk factors through various mechanisms, including regulation of blood pressure, metabolic function, gut barrier function, and gut microbiota activity [163]. Butyrate is the most extensively studied SCFA and numerous beneficial effects have been reported for CMD risk factors [164-166]. Lower butyrate in the gut has been linked with development of CVD and metabolic syndrome [167]. In regulation of blood pressure, butyrate can lower diastolic blood pressure by reduced inflammation [168]. Butyrate also inhibits activation of NF-κB, which plays a role in immune responses such as IL-6, TNF-α, and IL-1β production [169]. Acetate affects cholesterol and lipogenesis metabolism, and plays a direct role in regulation of appetite [28, 170, 171]. Propionate is gluconeogenic and is metabolized

in the liver. Propionate and butyrate may stimulate satiety signals through interaction with gut fatty acid receptors FFAR3 [172] and may act as an effective inhibitor of lipid synthesis [173]. Several studies have shown that acetate and propionate also exhibit immune-suppressive activity and have anti-inflammatory properties [174, 175].

2.5 THE EFFECTS OF COMMON DIETARY SUGARS ON CARDIOMETABOLIC RISK FACTORS AND INFLAMMATION

The simplest form of carbohydrates, also known as 'sugars', conventionally comprises monosaccharides and disaccharides [6, 7]. In brief, monosaccharides are sugar molecules consisting of glucose, fructose, and galactose that are found in a variety of natural food sources, including fruits, vegetables, and dairy products. In general, sugars have a sweet taste and are colorless and water-soluble. Glucose is the most abundant monosaccharide and a major source of energy in most organisms [7]. Metabolism of glucose in cells through glycolysis provides 4 calories per gram of carbohydrate. Fructose is another common monosaccharide, largely found in fruits and commonly present from hydrolysis of sucrose. Fructose is sweeter than glucose and is used commercially in the beverage and food industries. Fructose differs from glucose in several significant ways, including absorption and metabolism, which mainly occur in the liver [176]. Galactose is commonly found in dairy products, as a building block in the disaccharide lactose, and may be incorporated into galactolipids, which are structural components of the central nervous system in the body [177, 178].

Disaccharides are two sugar molecules linked by a glycosidic linkage. Common examples are sucrose, lactose, and maltose, which are widely found in food products. Sucrose is a dominant disaccharide, also known as 'table sugar', composed of glucose and fructose molecules. It is refined from sugar cane and sugar beet, and has been widely used as a sweetener for centuries [6]. Lactose is made up of glucose and galactose molecules and is mainly present in dairy products, while maltose is a combination of two glucose molecules produced in hydrolysis of starch. Polyols, sugar alcohols, are also classified as sugars. Polyols are naturally produced and commercially synthesized, and are used as sweeteners such as xylitol, mannitol, and sorbitol [6].

High intake of simple sugars has been postulated to contribute to inflammation processes by altering proinflammatory mediators through various mechanisms [87]. Many studies have reported an association between excessive intake of simple sugars and inflammation [3, 21, 22]. A pro-inflammatory condition such as hyperglycemia is associated with high glucose uptake, and may induce oxidative stress and exacerbate vascular cell damage [66]. Increased expression of plasma cytokines such as IL-6 and TNF-α, adhesion factors, e-selectin, intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and IL-8 has been observed in individuals with acute increases in blood glucose. This acute inflammatory response normalizes within 2-3 hours. However, T2D subjects and individuals with impaired

glucose tolerance demonstrate stronger and longer expression of inflammatory responses. T2D subjects also show increased expression of ROS and increased oxidation of LDL [179, 180].

2.5.1 Effects of high fructose and high galactose intake on cardiometabolic risk factors and inflammation

Fructose

High fructose or high galactose intake is believed to contribute to development of metabolic disorders. Decades ago, fructose was used as an alternative to other simple sugars, particularly in T2D patients, since it improved glucose response in a dose-dependent manner [181]. However, controversy arose when it was discovered that fructose intake favors lipidemia.

Metabolism of fructose produces various metabolites and byproducts such as lactate, glucose, free fatty acids, and uric acid, which can directly or indirectly disturb tissue and organ functions [182]. Impairment of β-cell mass has been observed in rats fed high-fructose diets, while high-fructose diets have also been associated with insulin resistance, dyslipidemia, and inflammation [183, 184]. In addition, high frutose intake may cause hyperlactatemia, which may result in insulin resistance through impairment of insulin signaling by preventing the activity of phosphatidylinitisol-3-kinase (P13K) and protein kinase B [185].

Both human and animal studies have found unfavorable effects of higher consumption of fructose, *e.g*., in development of metabolic syndrome and chronic diseases [23, 186-188]. Emerging evidence indicates a close relationship between metabolic syndrome induced by fructose and inflammation [19, 189]. Adipose tissue, a key site for energy homeostasis, shows increased expression of proinflammatory cytokines in the circulation after high fructose intake [190]. Excessive intake of fructose at physiological dosages may also induce inflammatory changes in vascular cells by increasing serum intercellular adhesion molecule-1 (ICAM-1) concentration, pro-inflammatory cytokines including IL-6, IL-10, and TNF-α, protein mediators, and recruitment of inflammatory cascades during inflammation [19, 22]. In addition, high fructose intake has been associated with intestinal barrier dysfunction [191]. Endotoxemia due to translocation of LPS can result in metabolic syndrome, including chronic inflammation, insulin resistance, and non-alcoholic fatty liver disease (NALFD) [192].

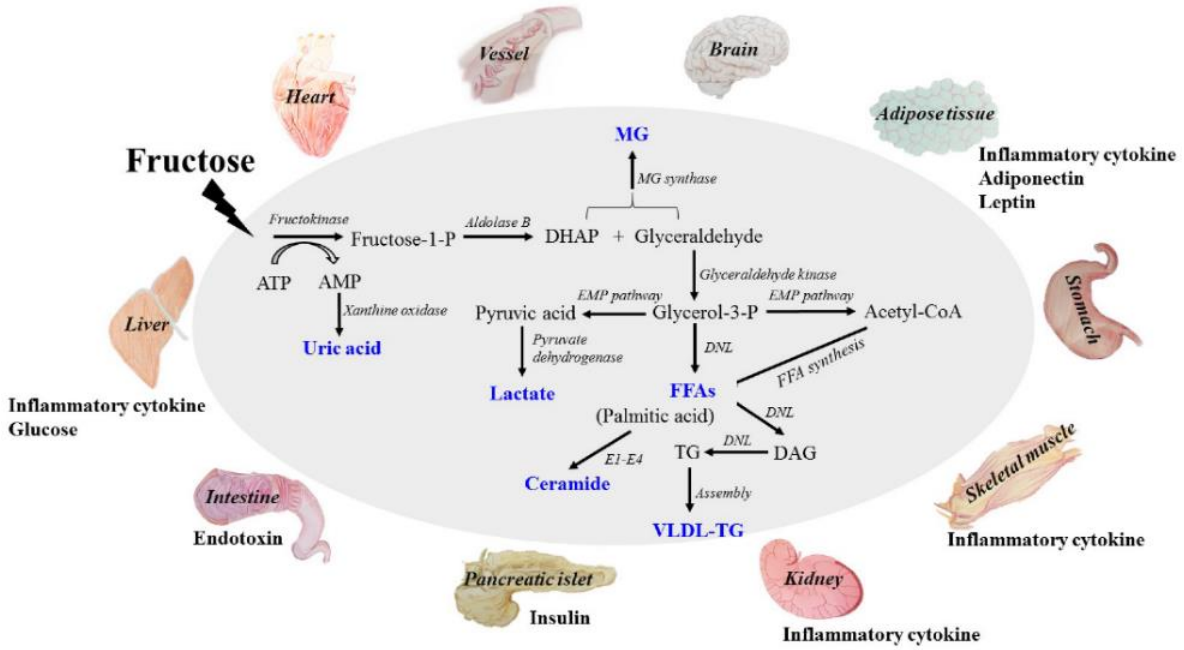

Figure 4. Overview of fructose metabolites and direct and indirect effects of high fructose intake on tissue and organ functions (source: Zhang *et al.*[182]).

Galactose

Many studies of galactose have focused on its genetic-related link to metabolic diseases, such as galactokinase- and galactose-1-phosphate uridyltransferase deficiency [21, 193]. Galactose is primarily metabolized into glycogen in the liver. Metabolism of galactose into glycogen could lower integration of glucose into glycogen, due to lack of hepatocytes to store the glycogen. However, consumption of a diet with more than 30% galactose may result in hypergalactosemia because of inefficient uptake of galactose in hepatic cells [194]. Galactose intake at higher levels has been linked to cataract formation in humans and animals, due to accumulation of galactitol in the lens [177, 195].

A high concentration of D-galactose and other reducing sugars may induce production of AGEs and ROS [11]. AGEs are produced in the Maillard reaction, which is a spontaneous reaction between reducing sugars and free amines such as the amino side-groups of amino acids [65, 196, 197]. Accumulation of AGEs enhances IL-1β secretion via inflammasome, which is independent of any other metabolic changes. In animal studies, continuous injection of D-galactose produces ROS and AGEs and leads to pathological processes of age-related diseases, such as diabetes, atherosclerosis, and Alzheimer's disease [63, 198, 199]. Several human studies have consistently reported that diets with high AGEs may enhance inflammation, oxidative stress, and biomarkers of endothelial function [196] leading to CVD, CKD, and diabetes. The pathological cascades of AGEs have been linked to activation of NF-κB and generation of pro-inflammatory factors such as TNF-α and IL-1β, as well as ROS [199, 200].

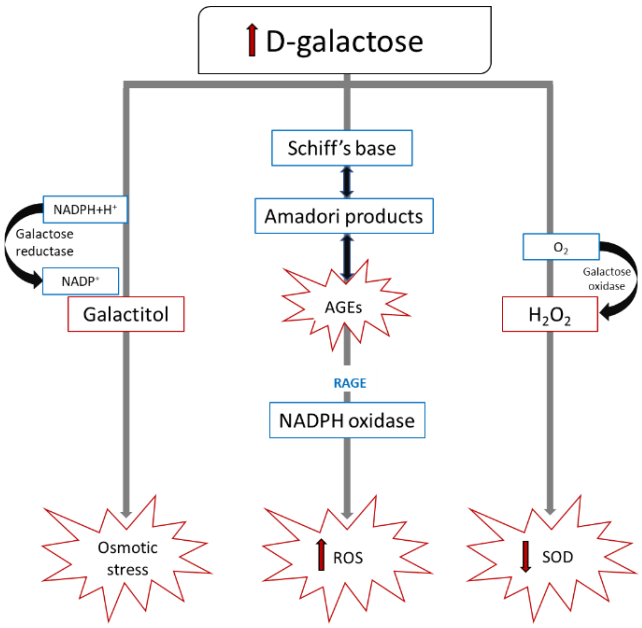

Figure 5. Summary of adverse effects after high galactose intake (modified from Bo-Htay *et al.* [201]). ROS – reactive oxygen species, SOD – superoxide dismutase.

3 AIMS

The overall aim of this thesis was to investigate the impact of dietary carbohydrate quality indicators such as whole grains, sugars, and fermentable dietary fiber on selected cardiometabolic risk factors, with emphasis on low-grade systemic inflammation.

Specific objectives were to:

1. Investigate the association between whole grain consumption, assessed by food frequency questionnaires (FFQ) and by concentration of alkylresorcinols in plasma and in adipose tissue, as a biomarker of whole grain intake, and selected biomarkers of inflammation and cardiometabolic risk factors in men and women (**Paper I**).

2. Evaluate alkylresorcinol concentrations in adipose tissue biopsies as a biomarker of long-term consumption of whole grain wheat and whole grain rye in free-living Swedish men and women (**Paper II**).

3. Explore the effects of high whole grain and bran rye consumption on low-grade inflammation after a six-week dietary intervention study in men with prostate cancer (**Paper III**).

4. Investigate the effect of high fructose or high galactose intake, with and without added fructooligosaccharides, on advanced glycation end products, metabolic factors, inflammation, and gut barrier function in a rat model (**Paper IV**).

5. Investigate the effect of high fructose or high galactose intake, with and without added fructooligosaccharides, on gut microbiota composition in a rat model (**Paper V**).

4 SUBJECTS AND METHODS

4.1 STUDY DESIGNS

Three different types of studies were used to address the specific aims of the thesis: (i) a human observational study with a cross-sectional design (**Papers I** and **II**); (ii) a human experimental/intervention study (**Paper III**); and (iii) an animal experimental study using a rat model (**Papers IV** and **V**). A summary of the work is presented in **Figure 6.**

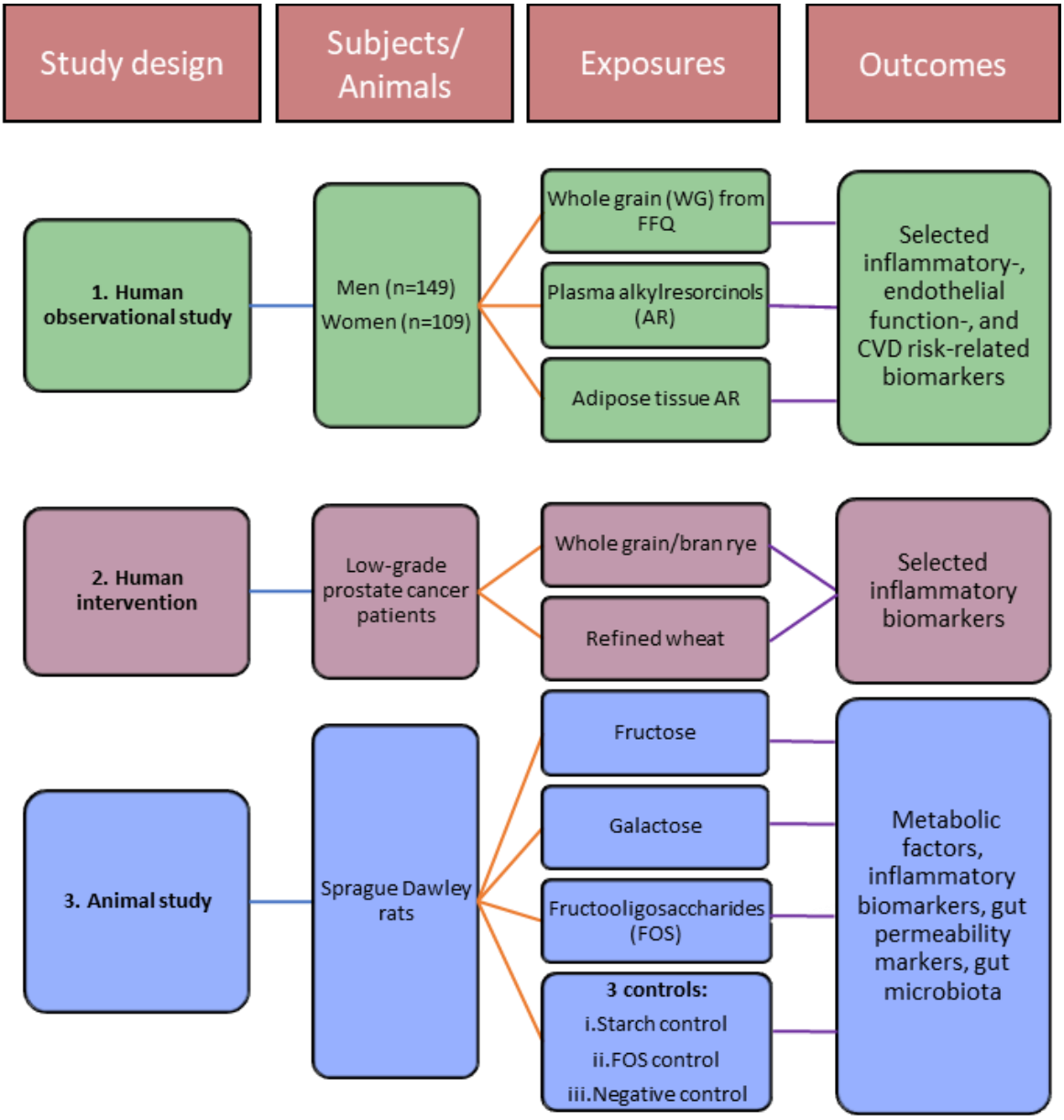

Figure 6. Overview of the studies in the thesis. Green – **Papers I** and **II**, pink – **Paper III**, blue – **Papers IV** and **V**. FFQ – food frequency questionnaire, CVD – cardiovascular disease.

4.1.1 Swedish Mammography Cohort–Clinical and Cohort of Swedish Men–Clinical

The observational studies (**Papers I** and **II**) were based on data and samples obtained from women and men in two population-based cohort studies, the Swedish Mammography Cohort (SMC) and the Cohort of Swedish Men (COSM). Data on the long-term diet of the subjects were collected using food-frequency questionnaires (FFQs), the design of which is shown in **Figure 7**.

The SMC was initiated in 1987 and involved women who were born between 1914 and 1948 and residing in Västmanland and Uppsala counties, Sweden. The invitation to the mammography screening was sent together with a questionnaire on diet (67-food item FFQ), weight, height, marital status, and education (FFQ-87), and the response rate was 74%. In 1997, a more comprehensive questionnaire was sent to all participants who were still alive. It included 96 food items, with additional questions regarding dietary supplement use, smoking status, and physical activity (FFQ-97), and the response rate was 70%. Between 2003 and 2009, the SMC participants who resided in Uppsala county were invited to participate in a sub-cohort study, the so-called SMC-Clinical (SMC-C) (**Figure 7**). The women were asked to complete a 123-item FFQ (FFQ-03), undergo a health examination including measurement of body weight, height, hip and waist circumference, blood pressure, and dual-energy X-ray absorptiometry scan, and donate biosamples. The biosamples included fasting blood, adipose tissue biopsies, and urine, and the response rate in SMC-C was 60%. In **Papers I** and **II**, biosamples from 109 women randomly selected from among over 5000 participants in the SMC-C were used.

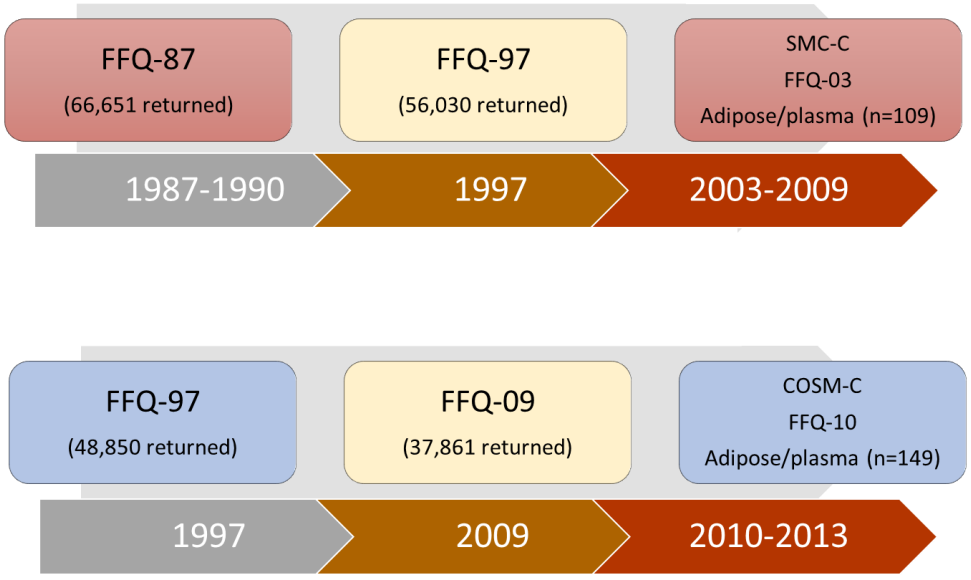

Figure 7. Overview of repeated dietary data collection by food frequency questionnaire (FFQ) reflecting long-term consumption in women from the Swedish Mammography Cohort-Clinical (SMC-C) and in men from the Cohort of Swedish Men-Clinical (COSM-C) (data used in **Papers I** and **II)**.

The COSM was initiated in 1997 and involved men who were born between 1918 and 1952 and residing in Örebro and Västmanland counties. Participants were required to complete a questionnaire FFQ-97, the same as in the SMC 1997, and response rate was 50%. In 2009, another questionnaire (FFQ-09) was sent to all participants still residing in the study area. Between 2010 and 2019, the COSM participants living in Västmanland county were invited to participate in a sub-cohort study, the so-called COSM-Clinical (COSM-C) (**Figure 7**). The men were asked to complete 132-item FFQ (FFQ-10) and undergo a clinical examination, including measurement of body weight, height, hip and waist circumference, and blood pressure. They donated blood, adipose tissue, and urine samples according to the same protocol as in the SMC-C. In **Papers I** and **II,** blood and adipose tissue biopsy samples from 149 men randomly selected from among over 5000 participants in the COSM-C were used.

4.1.2 Whole grain intake

Whole grain intake was estimated from three FFQs: in women FFQ-87, FFQ-97, and FFQ-03 and in men FFQ-97, FFQ-09, and FFQ-10 (**Papers I** and **II**). The average food intake values (g/day) for crispbread, whole-meal bread, white bread, porridge, muesli, pasta, and pancakes were converted from self-reported frequency of intake (range 3/day, 2/day, 1/day, 5-6/week, 3-4/week, 1-3/month, or never) and multiplied by age-specific portion size [(202)]. The average content of whole grain rye and whole grain wheat in cereal products on the Swedish market was calculated using the Swedish National Food Agency database. The value obtained was multiplied by daily product intake from FFQs, to obtain the estimates of whole grain intake (g/day). In brief, mean WG rye intake, calculated from FFQ-97, FFQ-09, and FFQ-10 for men and from FFQ-87, FFQ-97, FFQ-03 for women, was 43.4 g/d for men and 29.4 g/d for women. The corresponding figure for WG wheat intake was 20.6 g/d and 13:0 g/d, respectively.

In **Paper I**, whole grain rye and wheat (WGRnW) intake was estimated by combining whole grain rye and whole grain wheat FFQ-based intakes. Whole grain rye to whole grain rye and wheat intake ratio (WGR/WGRnW) was also calculated. In **Paper II**, various FFQ-based intakes, comprising WG products (WGP), total WG (TWG), WG wheat (WGW), WG rye (WGR), and WG rye and wheat (WGR&W), were calculated as the mean of three repeated dietary assessments in men (FFQ-97, FFQ-09, FFQ-10) and in women (FFQ-87, FFQ-97, FFQ-03).

4.1.3 Alkylresorcinols

Alkylresorcinol concentrations were measured in plasma and in adipose tissue of participants from the SMC-C and COSM-C (**Papers I** and **II**), according to a method described by Wierzbicka *et al*. [(119)].

4.1.4 A whole grain/rye bran intervention study in men with prostate cancer

Samples and data used in **Paper III** were from 17 men with clinically diagnosed prostate cancer, defined by Gleason score and tumor stage based on tumor spread to lymph nodes and metastasis staging system [(203)]. All participants had low-risk prostate cancer, with a Gleason score of 5, and participated in the study on an outpatient basis. Their mean age was 73±4.6 y, BMI was 27.5±4.6 kg/m^2, and all men were non-smokers and had no intake of dietary supplements. The study was conducted as a randomized, non-blind cross-over design with six-week interventions separated by a two-week washout period (**Figure 8**). Participants received both the intervention diets, and blood samples were collected before and after 2, 4, and 6 weeks of the treatment [(204)].

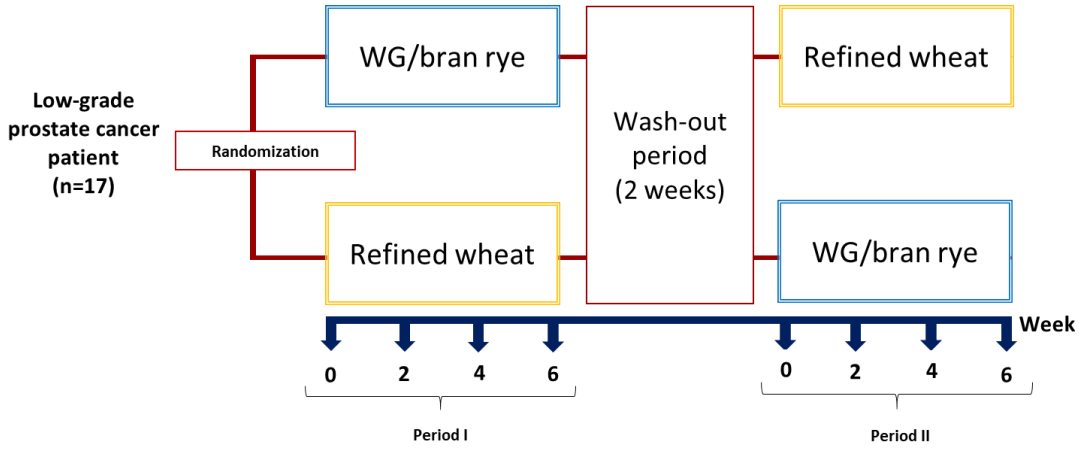

Figure 8. Overview of the human intervention study in **Paper III.** WG – whole grain.

All participants received WG/bran rye products (RP) and refined wheat grain products (WP) with added cellulose. The intervention food and macronutrient content in **Paper III** is presented in **Table 1**. All participants were advised to keep to their regular diet and consume all intervention foods (keep leftovers, if any) as part of their breakfast, lunch, and dinner. No cereal-based products such as bread, porridge, and table spread were allowed during the intervention [(204)].

Table 1. Summary of whole grain/bran product intake and macronutrient content per day

	Intervention food (per day)
Breakfast	300 g soft bread (3 pieces)
Lunch	100 g crispbread (10 pieces)
Dinner	50 g breakfast cereals (muesli)
2 to 3 snacks	33 g porridge (uncooked)
	58 g table spread

Macronutrient (g/d)	*RP*	*WP*
Carbohydrate	134±16	135±23
Protein	52±5	49±8
Fat	36±5	34±5
Fiber	77±9	76±1

The intervention foods for this study were designed and supplied by Lantmännen AB and WasaBröd, Sweden.

4.1.5 Experimental study in rats

The rat study (**Papers IV** and **V**) was performed at the Institute of Nutritional Sciences, University of Hohenheim, Germany. Healthy male Sprague Dawley rats (n=90) were purchased from Janvier Labs at seven weeks of age, with body weight range 250-274 g. The rats were housed at 20-22 °C in a humidity-controlled (50±10%) animal room with a 12-h light/12-h dark cycle, and provided with food and water *ad libitum*. All rats were fed a starch diet (control) for a two-week adaptation period before the experiment started.

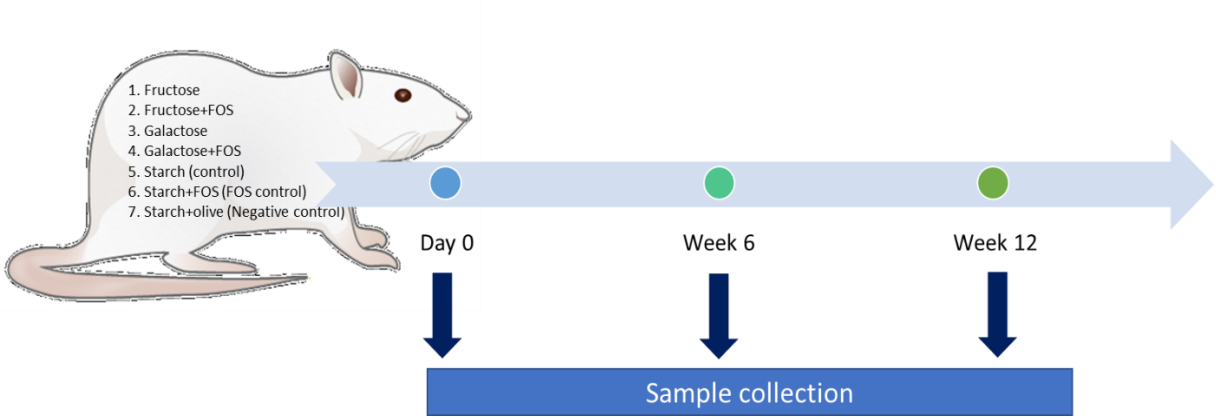

Figure 9. Overview of the experimental animal study in **Papers IV** and **V**. FOS – fructooligosaccharides.

The rats were randomly divided into seven treatment groups (n=12 for each group) and one baseline group (n=6) to measure the metabolic status of the rats before the experiment. In brief, the rats were grouped by different type of intervention diets as follows: 1) fructose, 2) fructose+FOS, 3) galactose, 4) galactose+FOS, 5) starch (control), 6) starch+FOS (FOS control), and 7) starch+olive (negative control). Starch was used as a control and was added to all intervention diets to obtain isocaloric conditions. Starch with added FOS was used as a control to evaluate the effect of FOS during a 12-week intervention. Starch with olive oil as part of fat was used as a negative control, to determine the effect of safflower oil, which contains higher concentrations of n-6 polyunsaturated fatty acids (n-6 PUFA), inducing low-grade inflammation. The baseline group was sacrificed at day 0 and then six rats from each group were sacrificed at 6 weeks and another six rats at 12 weeks (**Figure 9**). The rats were fasted for 12 hours and anesthetized with carbon dioxide gas before decapitation. Blood samples from each rat were collected into heparinized monovettes (Monovette®, Sarstedt, Germany), and samples of feces and small and intestinal contents were collected in Eppendorf tubes. Tissues, including liver, kidney, and brain, were kept in cryotubes and snap-frozen in liquid nitrogen. All samples were immediately stored at -80 °C prior to analysis.

4.1.6 Fructose and galactose

Diets rich in simple sugars, particularly high fructose and high galactose, were formulated for **Papers IV** and **V** based on a review of the literature reporting effects on low-grade inflammation within the suggested dosing range [205-208]. In brief, each intervention diet contained either 50% kcal from fructose (50g/100g) or 50% kcal from galactose (50g/100g), except for the control groups (**Table 2**). The high dose was chosen to increase the likelihood of some galactose and fructose escaping digestion in the small intestine and reaching the colon, in order to evaluate the potential impact on gut microbiota.

4.1.7 Fructooligosaccharides (FOS)

FOS used in this study was inulin-type from chicory root with 93-97% purity (Orafti® Oligofructose, Boneo GmbH, Germany). In total, 7.9% of the daily energy intake derived from FOS (15g/100g) for those diets with added FOS (**Papers IV** and **V**) (**Table 2**). The amount of FOS was selected based on reported doses shown to reverse potential pro-inflammatory response in rats [209, 210].

Table 2. Dietary composition of the high carbohydrate diet

	Starch (Control)	Fructose	Galactose	Starch+FOS (FOS Control)	Fructose+FOS	Galactose+FOS	Starch+olive (Negative Control)
Energy, kcal	3970	3970	3970	3820	3820	3820	3970
Carbohydrates							
Starch, g/kg	615	115	115	540	40	40	615
Fructose, g/kg	-	500	-	-	500	-	-
Galactose, g/kg	-	-	500	-	-	500	-
% Energy	62.0	62.0	62.0	56.5	56.6	56.6	62.0
FOS, g/kg	-	-	-	150	150	150	-
Cellulose, g/kg	125	125	125	50	50	50	125
% Energy	6.3	6.3	6.3	10.5	10.5	10.5	6.3
Protein							
g/kg	180	180	180	180	180	180	180
% Energy	18.1	18.1	18.1	18.9	18.9	18.9	18.1
Fat:							
Safflower oil, g/kg	60	60	60	60	60	60	-
Olive oil, g/kg	-	-	-	-	-	-	60
% Energy	13.6	13.6	13.6	14.1	14.1	14.1	13.6
Minerals & vitamins							
g/kg	20	20	20	20	20	20	20

FOS – fructooligosaccharides.

4.2 SELECTED BIOMARKERS ANALYZED AS OUTCOMES IN THE DIFFERENT STUDIES

Different metabolic, endothelial function, inflammatory, gut function and CVD biomarkers were selected and analyzed in **Papers I, III, IV,** and **V**, based on markers previously identified as being altered by whole grain, dietary fiber, or sugar intake in dietary intervention studies reported in the literature. The biomarkers used are listed in **Table 3**.

Table 3. Summary of selected biomarkers measured in plasma in **Papers I, III, IV,** and **V**

	Paper I	**Paper III**	**Paper IV and V**
Biomarkers	*Inflammatory markers*	*Inflammatory markers*	*Inflammatory markers*
	hsCRP	IL-1Ra	CRP
	IL-1Ra	IL-1α	TNF-α
	TNF-R1	IL-6	IL-1β
	TNF-R2	TNF-R2	IL-6
		92-inflammation related protein (OLINK)	
	Endothelial markers		*Metabolic factors markers*
	ICAM-1	*Endothelial markers*	Blood glucose
	VCAM-1	E-selectin	Insulin*
	E-selectin	ICAM-1	Total cholesterol
		VCAM-1	HDL
	CVD-related markers		LDL
	Endostatin	*CVD-related markers*	Triglycerides
	Cathepsin S	Cathepsin S	
	Cathepsin B	Endostatin	*Inflammation-related markers*
			CML
			Pentosidine
			Lysine
			Gut permeability markers
			Zonulin
			Endotoxin
			Liver function markers
			AST
			ALT
			ALP
			Kidney function markers
			Urea
			Creatinine
			Uric acid

*Analyzed in serum. ALP – alkaline phosphatase; ALT – alanine aminotransferase; AST – aspartate transaminase; CML – Nε-(carboxymethyl)lysine; CRP – c-reactive protein; HDL – high density lipoprotein; hsCRP – high sensitivity c-reactive protein; ICAM-1 – intercellular adhesion molecule-1; IL-1β – interleukin-1β; IL-1Ra β – interleukin-1 receptor antagonist; IL-6 – interleukin-6; LDL – low density lipoprotein; TNF-α – tumor necrosis factor-α; TNF-R1 – tumor necrosis factor-receptor 1; TNF-R2 – tumor necrosis factor-receptor 1; VCAM-1 – vascular cell adhesion molecule-1.

Biomarkers in samples from subjects in the SMC-C and COSM-C (**Paper I**) were measured using commercial sandwich enzyme-linked immunosorbent assays (ELISA). High sensitivity c-reactive protein (hs-CRP) was measured using a Mindray BS 280 Instrument (Mindray, Shenzhen, China). Biomarkers from low-grade prostate cancer patients (**Paper III**) were also measured using commercial sandwich enzyme-linked immunosorbent assays (ELISA), while 92 inflammation-related protein biomarkers were analyzed using a multiplex immunoassay (Olink Bioscience, Sweden). Biomarkers from the rat studies (**Papers IV** and **V**) were analyzed using

specific methods, *e.g.*, ELISA, colorimetric methods, and analytical methods [211], depending on the specific marker.

4.3 GUT MICROBIOTA

Gut microbiota composition in large intestine contents collected at baseline, six weeks, and 12 weeks was analyzed using the 16S rRNA method (**Figure 10**) (**Paper V**).

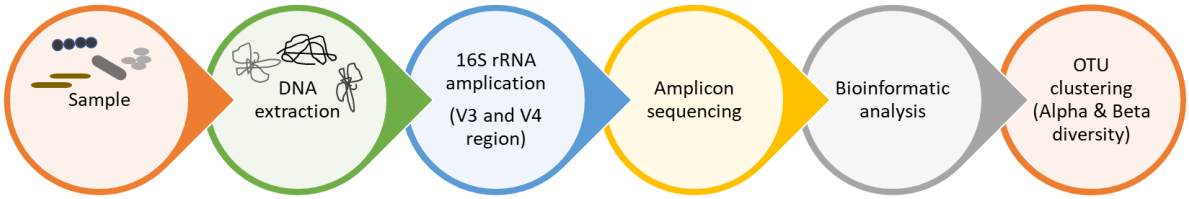

Figure 10. Workflow of gut microbiota analysis.

Deoxyribonucleic acid (DNA) from microbiota samples was extracted using the QIAamp Fast DNA Stool mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The v3 and v4 regions of the 16S rRNA gene were amplified using the primers 341F and 805R. Polymerase chain reaction (PCR) was carried out using Phusion® High-Fidelity PCR Master Mix (New England Biolabs) and PCR products were purified using Qiagen Gel Extraction Kit (Qiagen) and quantified with Qubit®3.0 Fluorometer (Invitrogen, Thermo Fisher Scientific). The NEBNext® UltraTM DNA Library Prep Kit was used to generate the final libraries. The amplicons were sequenced on the Illumina platform at Novogene (Beijing, China).

4.4 STATISTICAL ANALYSES

All analyses were carried out using Statistical Analysis System, version 9.3/9.4 (SAS Institute, Cary, NC, US) (**Papers I-V**). Principal component analysis (PCA) was conducted using the open-source statistical environmental RTeam with in-house scripts (**Paper III**). A summary of the statistical methods used in **Papers I-V** is presented in **Table 4**. The assumption of normality and homogeneity of variance for all datasets of each study was tested using the Shapiro-Wilks test. Two-sided $P<0.05$ was considered statistically significant.

Table 4. Summary of statistical methods, dependent and independent variables, and covariates used in the studies reported in **Papers I-V**

Paper	Statistical procedure (SAS)	Dependent variables	Independent variables[1]	Covariate
I	**Student's *t*-test** **ANCOVA**	Biomarkers	Whole grain intake (WGRnW and WGR/WGRnW) from FFQs (COSM-C: FFQ-97, FFQ-09, FFQ-10; SMC-C: FFQ-87, FFQ-97, FFQ-03) Total AR and C17:0/C21:0 ratio in plasma and adipose tissue	BMI, age, and sex
II	**CORR** **%ICC[5]** **Means** **ANOVA** **GLM**	SMC-C[3]: AR concentration in plasma and adipose tissue	SMC-C: Whole grain intake (FFQ-87, FFQ-97, FFQ-03)	BMI, age, and sex
		COSM-C[4]: AR concentration in plasma and adipose tissue	COSM-C: Whole grain intake (FFQ-97, FFQ-09, FFQ-10)	
III	**Mixed linear model (Proc mixed)** **CORR** **GLM** **PCA**	Biomarkers	Diet, sequence, period, *subject*	Occasion, baseline value
IV	**ANOVA[2]**	Biomarkers	Diets	Baseline value
V	**ANOVA[2]**	Gut microbiota (phylum and genus level)	Diets	
	CORR	Biomarkers	Gut microbiota (phylum)	

[1]Random factors in italics. [2]Tukey's pair-wise comparison was used as *post hoc* test.[3]Alkylresorcinols (AR) in plasma and adipose tissue were collected in 2003. [4]AR in plasma and adipose tissue were collected in 2010. [5]Intra-class correlation coefficient.

5 RESULTS AND DISCUSSION

5.1 LONG-TERM WHOLE GRAIN INTAKE, BIOMARKERS OF WHOLE GRAIN INTAKE, AND LOW-GRADE INFLAMMATION IN OBSERVATIONAL STUDIES

5.1.1 Baseline characteristics of men and women in Papers I and II

The characteristics of participants included in the observational studies (**Papers I** and **II**) are summarized in **Table 5.** In total, 258 participants were included in the investigations; 149 men (from COSM-C) and 109 women (from SMC-C). On average, the men were older than the women (mean 87 y and 66 y, respectively), but no differences in BMI were observed (24.6 kg/m^2 and 24.3 kg/m^2, respectively).

Energy intake from the mean of the three FFQs (see **Figure 7**) was significantly higher in men (2314 Kcal/d) than in women (1644 Kcal/d). Average daily whole grain rye and wheat intake was also higher for men (WG rye; 43.4 g/d, WG wheat; 20.6 g/d) than for women (WG rye; 29.4 g/d, WG wheat; 13.0 g/d). As reported in previous studies, higher age was associated with higher intake of whole grain products [212]. Total whole grain intake was higher than that reported in the national dietary survey *Riksmaten*, where it was 39 g/d for women and 46 g/d for men [213].

Table 5. Biomarkers of inflammation, endothelial function, and cardiovascular disease (CVD) risk in men from the Cohort of Swedish Men-Clinical (COSM-C) and women from the Swedish Mammography Cohort-Clinical (SMC-C).

	Men (n=149)		Women (n=109)		*P*
Biomarkers	Mean	95% CI	Mean	95% CI	
Inflammatory					
hs-CRP (mg/L)	2.2	1.0, 3.9	1.4	0.7, 2.1	***
IL-Ra (µg/L)	0.2	0.2, 0.9	0.2	0.2, 0.9	NS
TNF-R1 (µg/L)	1.4	1.2, 1.9	1.1	1.0, 1.4	***
TNF-R2 (µg/L)	5.4	4.5, 6.7	5.2	4.4, 6.1	*
Endothelial function					
ICAM-1 (µg/L)	189.3	164.8, 228.0	149.6	132.4, 179.4	***
VCAM-1 (µg/L)	325.9	282.3, 399.1	282.3	250.3, 349.7	***
E-selectin (µg/L)	11.8	9.0, 14.9	12.3	9.1, 15.3	NS
CVD risk-related					
Cathepsin B (µg/L)	74.4	56, 6 95.2	50.1	38.5, 65.7	***
Cathepsin S (µg/L)	7.6	6.7, 8.7	9.1	8.0, 10.3	***
Endostatin (µg/L)	57.2	44.2, 69.6	55.1	47.4, 65.8	NS

CI – confidence interval ; NS – non-significant. Comparisons between men and women using Student t-test. *P<0.05, **P<0.01, ***P<0.001.

Overall, no differences were observed between men and women for AR concentrations in plasma, *i.e.*, total AR, AR homologs C17:0-C25:0, and C17:0/C21:0 ratio. However, AR concentrations in adipose tissue were significantly higher in men than women, but not C17:0/C21:0 ratio (**Table 6**). The range of AR concentrations recorded in adipose tissue were in line with values reported in previous studies [122, 123].

Table 6. Alkylresorcinols (AR) in plasma and adipose tissue in men from the Cohort of Swedish Men-Clinical (COSM-C) and women from the Swedish Mammography Cohort-Clinical (SMC-C)

	Men (n=149)		Women (n=109)		*P*
	Mean	95% CI	Mean	95% CI	
Alkylresorcinols in plasma					
Total AR (nmol/L)	51	31 , 82	47	31 , 71	NS
C17:0 (nmol/L)	6	3 , 11	5	3 , 10	NS
C19:0 (nmol/L)	17	11 , 28	15	9 , 25	NS
C21:0 (nmol/L)	18	11 , 27	17	12 , 25	NS
C23:0 (nmol/L)	5	3 , 8	5	3 , 8	NS
C25:0 (nmol/L)	4	2 , 8	6	3 , 9	NS
C17:0/C21:0	0.3	0.2 , 0.5	0.3	0.2 , 0.5	NS
Alkylresorcinols in adipose tissue					
Total AR (pmol/g)	1142	711 , 1755	883	565 , 1303	*
C17:0 (pmol/g)	108	52 , 162	76	46 , 111	*
C19:0 (pmol/g)	329	216 , 519	248	168 , 374	*
C21:0 (pmol/g)	398	249 , 612	308	204 , 474	*
C23:0 (pmol/g)	124	77 , 204	103	63 , 150	*
C25:0 (pmol/g)	149	85 , 257	116	81 , 199	*
C17:0/C21:0	0.3	0.2 , 0.3	0.3	0.2 , 0.4	NS

CI – confidence interval ; NS –non-significant. Comparison between men and women by Student t-test. **P*<0.05, ***P*<0.01, ****P*<0.001.

5.1.2 Associations between self-reported whole grain rye and wheat intake, alkylresorcinols as intake biomarkers, and inflammatory, endothelial function, and CVD-risk related markers (Paper I)

Among self-reported whole grain intake variables, only total WGRnW intake was statistically significantly associated with one CVD-related biomarker, namely cathepsin S (Q1: 7.8, Q4: 8.8, *P-trend* = 0.025). Cathepsin S is a lysosomal protease that is involved in degradation of damaged proteins through hydrolysis of peptide bonds of amino acids. Cathepsin S is involved in wide range of physiological processes, such as expression of cytokines and chemokines, cell signaling, apoptosis, and antigen presentation by MHC class II.

Loss of regulation of cathepsin S has been shown in development of various pathological conditions leading to diabetes, CVD, arthritis, and cancers [214]. Cathepsin S is upregulated in diabetic individuals compared with healthy individuals, and has become a therapeutic target to delay progression of diabetes [215]. Higher expression of cathepsin S has also been observed in adipose tissue in obese individuals, but has been shown to be lower after weight reduction, indicating that cathepsin S is influenced by the diet and improved by weight loss [216, 217]. Moreover, cathepsin S has been shown to be strongly associated with increased LDL and triglyceride concentration [216, 218]. A healthy Nordic diet has been shown to reduce cathepsin S concentration in slightly overweight and hyperlipidemic individuals, which could be due to reduction of weight and lower LDL concentrations [219]. The lack of association found for WGR/WGRnW ratio suggests that both WG rye and wheat were involved in this association.

In contrast to self-reported data, no significant associations were found for total AR and C17:0/C21:0 ratio in either plasma or adipose tissue. However, total AR (Q1: 61.7, Q4: 54.1, *P-trend* = 0.035) and C17:0/C21:0 ratio (Q1: 61.4, Q4: 54.4 , *P-trend* = 0.026) in plasma were inversely associated with expression of endostatin concentration, another CVD-related marker. No such association was found for AR in adipose tissue. Endostatin is a 20 kDa fragment cleaved from the C-terminal of collagen XVIII by proteolytic enzyme [220]. Endostatin is known to be an endogenous angiogenesis inhibitor and to be involved in cancer development and progression [221]. Anti-angiogenic functionality of endostatin plays an important role in inhibition of cancer cell migration, proliferation, and angiogenesis, and protects against growth and progression of tumors [220]. Moreover, expression of endostatin in circulation has been shown to have a positive association with cardiovascular phenotypes such as obesity, hypertension, kidney disease, and diabetes [222-224]. Higher concentrations of endostatins have also been associated with increased risk of CVD in patients with diabetes and kidney disease [222, 225] and with mortality [226].

Overall, the findings in **Paper I** may suggest that whole grain rye and wheat are associated with higher cathepsin S and lower endostatin concentrations. However, the associations appeared modest and might explain the inconsistency between the findings from FFQ and dietary biomarkers.

5.1.3 Alkylresorcinols in adipose tissue as biomarkers of long-term whole grain intake (Paper II)

The baseline characteristics of study participants included in **Paper II** were the same as for **Paper I (**described in section **5.1.1)**. Whole grain intakes and their stability over time, as estimated by intra-class correlation (ICC), in men and in women are presented in **Table 7**.

Overall, modest correlations were observed between self-reported whole grain intakes from FFQs and AR in adipose tissue. Correlations between mean intakes from the three FFQs and AR in adipose tissue were stronger when the average long-term intake based on repeated FFQs was calculated, than when using intake estimates from a single FFQ completed nearest to the AR adipose tissue measuring point. Correlation coefficient for whole grain intakes estimated over the period of 14 years (men) and 17 years (women) and AR in adipose tissue was in the range r=0.09-0.44, depending on specific whole grain intake variable: WGR (*r*=0.31-0.42) > WGRnW (*r*=0.27-0.44) > TWG (*r*=0.22-0.42) > WGP (*r*=0.15-0.36) > WGW (*r*=0.09-0.33). Whole grain rye intake was most strongly associated with adipose tissue AR, which could be due to regular consumption over time of WG rye, rich in AR, leading to accumulation. Whole grain wheat showed the weakest correlation with AR in adipose tissue among men, most likely due to irregular intake of WG wheat reported in FFQs, as supported by low ICC values in the range 0.10-0.28 in men (**Table 7)**. The findings of stronger correlations for estimated whole grain intakes (WGR, WGRnW, TWG), *i.e.*, g/d intake of WG as an ingredient of WG products, rather than of the WG product (containing WG), with adipose tissue AR concentrations confirm the benefits of estimating whole grain intake in g/d, rather than just reporting WG product intakes, in epidemiological studies. Moreover, a stronger correlation was observed between intake from repeated FFQs, rather than from a single FFQ, and AR concentrations in adipose tissues, suggesting that repeated FFQs improve accuracy in evaluation of long-term whole grain intake, at least in populations where the intake of whole grain does not vary substantially over the period.

On correlating whole grain intakes for men and women with individual AR homologs, it was found that WGRnW and WGR were significantly associated with AR homologs in adipose tissue, but not with AR homologs in plasma. This might be due to longer half-life of AR in adipose tissue than in plasma. However, WG wheat intake showed poor correlations with AR homologs in both adipose tissue and plasma. This might be due to large measurement errors inherent to estimation of WG wheat intake using FFQs. The C17:0/C21:0 ratio was around 0.30 in both plasma and adipose tissue, which suggests that both WG wheat and WG rye were consumed by the study participants, which is also in agreement with the self-reported intakes [(116)].

Table 7. Whole grain (WG) and energy intake (food frequency questionnaire (FFQ)-based) and intraclass correlation (ICC) of three repeated measurements in men and women

	Men (n=149)						Women (n=109)					
	FFQ-97		FFQ-09		FFQ-10		FFQ-87		FFQ-97		FFQ-03	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
FFQ-based (g/day):												
WGP	304	114	311	152	292	148	248	103	186	85	196	93
TWG	116	53	124	66	115	62	99	47	74	37	81	40
WGW	28	14	29	17	27	16	28	13	21	11	21	11
WGR	51	31	50	30	47	34	56	30	34	22	32	19
WGRnW	79	36	79	42	74	44	84	38	55	27	53	26
Energy (kcal/day)	2395	651	2436	709	2443	753	1625	411	1764	477	1742	502
	FFQ-97,09		FFQ-09,10		FFQ-97, 09, 10		FFQ-87,97		FFQ-97,03		FFQ-87, 97, 03	
	ICC	95% CI	ICC	95% CI	ICC	95% CI	ICC	95% CI	ICC	95% CI	ICC	95% CI
FFQ-based (g/day):												
WGP	0.31	(0.18, 0.47)	0.37	(0.24, 0.52)	0.31	(0.22, 0.42)	0.24	(0.10, 0.45)	0.52	(0.38, 0.65)	0.44	(0.34, 0.54)
TWG	0.36	(0.24, 0.51)	0.42	(0.30, 0.56)	0.39	(0.29, 0.49)	0.43	(0.29, 0.58)	0.54	(0.41, 0.67)	0.52	(0.43, 0.61)
WGW	0.28	(0.16, 0.45)	0.10	(0.02, 0.39)	0.14	(0.06, 0.28)	0.18	(0.06, 0.43)	0.44	(0.30, 0.59)	0.30	(0.21, 0.41)
WGR	0.46	(0.33, 0.58)	0.53	(0.42, 0.64)	0.48	(0.38, 0.57)	0.30	(0.16, 0.49)	0.46	(0.32, 0.60)	0.36	0.27, 0.47)
WGRnW	0.42	(0.30, 0.56)	0.40	(0.28, 0.54)	0.39	(0.30, 0.50)	0.25	(0.11, 0.46)	0.47	(0.33, 0.62)	0.34	(0.25, 0.45)
Energy (kcal/day)	0.31	(0.18, 0.47)	0.47	(0.35, 0.60)	0.34	(0.25, 0.45)	0.46	(0.32, 0.61)	0.53	(0.40, 0.66)	0.42	(0.33, 0.53)

WGP – whole grain products; TWG – total whole grains; WGW – whole grain wheat; WGR – whole grain rye; WGRnW – whole grain rye and wheat (source: modified from Wu *et al*. [227]).

In summary, AR in adipose tissue could be used as a biomarker of long-term WG rye intake after adjustment for sex, but it is still unclear whether it could also be used to reflect WG wheat intake. The correlations between AR in adipose tissue and estimated WG wheat intake from FFQ were poor, and further studies in which WG wheat is estimated using a more precise instrument are needed before conclusions can be drawn.

5.2 EFFECTS OF WHOLE GRAIN/BRAN RYE CONSUMPTION ON INFLAMMATORY, ENDOTHELIAL FUNCTION, AND CVD RISK-RELATED BIOMARKERS

Average total energy, dietary fiber, fat, and protein intake per day for the two intervention diets in 17 men with prostate cancer are presented in **Table 8**. The nine inflammatory biomarkers analyzed in **Paper III** are listed in **Table 3**.

Table 8. Summary of total energy and macronutrient intake per day during the dietary interventions

	RP	WP
Total energy (kJ/d)	8192±1175	8401±1989
Total dietary fiber intake (g/d)	24.5±8.0	23.0±10.2
Total fat intake (g/d)	76.1±17.5	61.0±12.3
Total protein intake (g/d)	86.8±16.7	88.0±16.1

RP – WG/bran rye products; WP – refined wheat grain products

After consumption of WG bran rye (RP) for six weeks, compared with refined wheat (WP) with added cellulose fiber, men had lower concentrations of TNF-R2, e-selectin, and endostatin (**Table 9**). No significant effects were found for the other biomarkers analyzed (cathepsin S, ICAM-1, IL-6, IL-Ra, VCAM-1). Several studies have reported that consumption of whole grains in the diet can reduce expression of inflammatory markers such as CRP, IL-6, and IL-1β in healthy individuals [125, 228]. More pronounced effects of WG diets in reducing proinflammatory cytokines such as CRP, TNF-R2, and ICAM-1 have been observed in unhealthy individuals such as in T2D patients [130, 228]. However, only a few studies have focused on the role of WG rye intake on such biomarkers, and in those studies consumption of WG rye had a greater effect on inflammatory biomarkers than consumption of WG wheat [204, 229].

Table 9. Effects of rye and wheat products in the diet on the tumor necrosis factor receptor 2 (TNF-R2), e-selectin, and endostatin

Parameter	Rye products	Wheat products	*P-value*
			Effect of diet
TNF-R2, ng/mL	4.7 (4.3 – 5.1)	5.3 (5.0– 5.6)	**0.023**
E-selectin, ng/mL	17.4 (16.5 – 18.4)	19.8 (18.8 – 20.8)	**0.001**
Endostatin, ng/mL	55.2 (50.4 – 60.5)	62.0 (59.0 – 65.2)	**0.018**

Data shown are least squares (LS) mean and 95% confidence interval (in brackets).

Men included in the study in **Paper III** had slow-growing prostate cancers and were under monitoring. In general, low-grade prostate cancer progresses slowly and is less likely to spread. Prostate-specific antigen (PSA) is a common molecular marker used for screening and surveillance of diagnosed prostate cancer [(230)]. Higher expression of PSA in the circulation is used to evaluate the presence and progression of prostate cancer, although the levels can also increase for other reasons [(230)].

In **Paper III**, the association between PSA and the selected biomarkers was measured (**Table 2** in **Paper III**). Partial correlation analysis revealed an inverse association between PSA concentration and ICAM-1 (r=-0.21, $P<0.05$), whereas PSA was positively correlated with endostatin (r=0.26, $P<0.01$). Apart from adhesion molecules, ICAM-1, a member of the immunoglobulin superfamily, also plays a role in stimulation of T-cells and natural killer cells [(231)]. Expression of ICAM-1 is reported to enhance antitumor responses and suppress the growth of prostate tumor [(232)]. In cancer patients, angiogenesis leads to progression of cancer cell growth, metastasis, and invasion. Anti-angiogenic therapy is a promising strategy to arrest the development of new blood vessels in cancer patients [(220, 233)]. Higher expression of endostatin has been observed in several cancers, which could be due to invading cancer cells cleaving endostatin from collagen XVIII [(220, 221)].

In **Paper III**, correlations were observed between cathepsin S concentration and endostatin (r=0.50, $P<0.001$), TNF-R2 (r=0.40, $P<0.001$) and e-selectin (r=0.33, $P<0.001$). ICAM-1 was significantly associated with VCAM-1 over treatment periods. Upregulation of ICAM-1 and VCAM-1 has been detected in various malignant tumors and they are reported to be involved in metastasis, *e.g.*, VCAM-1 transmigrates tumor cells into sub-epithelium and develops new niches [(231)]. Expression of the IL-1 family, particularly IL-1a, IL-1Ra, or IL-1RII, can be seen in patients with prostate cancer and benign prostatic hyperplasia patients [(234, 235)]. Upregulation of these markers is also associated with invasion of tumor and metastasis [(236)]. Explorative analysis of the effects of rye and wheat products on 92 inflammation-related proteins showed no significant effects (**Paper II**I).

Overall, the results in **Paper III** suggest that WG/bran rye consumption could lower expression of some inflammatory biomarkers, particularly endostatin, TNF-R2, and e-selectin concentrations, in low-grade prostate cancer patients. However, further intervention studies on the roles of WG/bran rye in modulation of subclinical inflammation in a large population are needed to confirm these findings.

5.3 EFFECTS OF HIGH FRUCTOSE AND HIGH GALACTOSE INTAKE, WITH OR WITHOUT ADDED FRUCTOOLOGOSACCHARIDES, IN RATS

The primary outcome of **Paper IV** and **V** was the effect of diets on metabolic factors, inflammation, gut integrity and gut microbiota composition after 12 weeks of intervention.

5.3.1 Energy intake and body weight

Energy intake (kcal/day) was higher in rats fed the fructose diet than in those fed the fructose+FOS and starch+FOS (FOS control) diets. The FOS intake range in the starch+FOS (FOS control), fructose+FOS, and galactose+FOS groups was 3.5 ± 0.1 g to 4.5 ± 0.2 g per day. The fructose intake in the fructose and fructose+FOS groups was 15.2 ± 0.4 g and 12.9 ± 0.3 g per day, respectively. The galactose intake from the galactose and galactose+FOS diets was 14.0 ± 0.7 g and 14.9 ± 0.8 g per day, respectively (**Table 10**). Even though energy intake was higher in rats fed the fructose diet, no difference was observed in botdy weight of rats in this group compared with controls.

Table 10. Summary of energy intake (kcal/day) and specific intake (g/day) of fructose, galactose, and fructooligosaccharides (FOS) in rats in specific intervention groups

		Dietary intake (g/day)		
Diet	**Energy intake (kcal/day)**	**Fructose**	**Galactose**	**FOS**
Starch	380.3 ± 8.2 ^{ab}	n.a	n.a	n.a
Starch+olive	369.5 ± 9.4 ^{abc}	n.a	n.a	n.a
Starch+FOS	318.6 ± 14.7 ^{c}	n.a	n.a	3.5 ± 0.1
Fructose	426.2 ± 9.9 ^{a}	15.2 ± 0.4	n.a	n.a
Fructose+FOS	350.2 ± 8.9 ^{bc}	12.9 ± 0.3	n.a	3.9 ± 0.1
Galactose	393.8 ± 19.9 ^{ab}	n.a	14.0 ± 0.7	n.a
Galactose+FOS	403.6 ± 20.8 ^{ab}	n.a	14.9 ± 0.8	4.5 ± 0.2

n.a. – not applicable.
Starch = control, starch+FOS = FOS control, starch+olive = negative control. Energy intake is expressed as least squares (LS) mean ± SEM for six rats, assessed by one-way ANOVA followed by Tukey's post hoc test. LS means with different superscripts (lowercase letters) are significantly different (P<0.05). Dietary intake is expressed as mean+SEM for six rats.

Long-term intake of high fructose may increase body weight and abdominal fat [237]. Several studies have suggested that a diet containing high fructose increases body weight due to a reduction in insulin and leptin and elevated ghrelin concentrations, which may result in overeating by disruption of satiety [183, 237]. In contrast, in **Paper IV** there was no difference in body weight between rats fed the high-fructose diet compared with the starch (control) and starch+olive (negative control) groups. These findings are in line with results in several previous studies demonstrating that high intake of fructose during interventions lasting eight weeks to seven months did not directly increase body weight, but increased abdominal fat and modulated blood lipids [238, 239]. However, abdominal fat was not measured in **Paper IV**, so it is not possible to support or refute previous findings on increased body fat after a fructose diet.

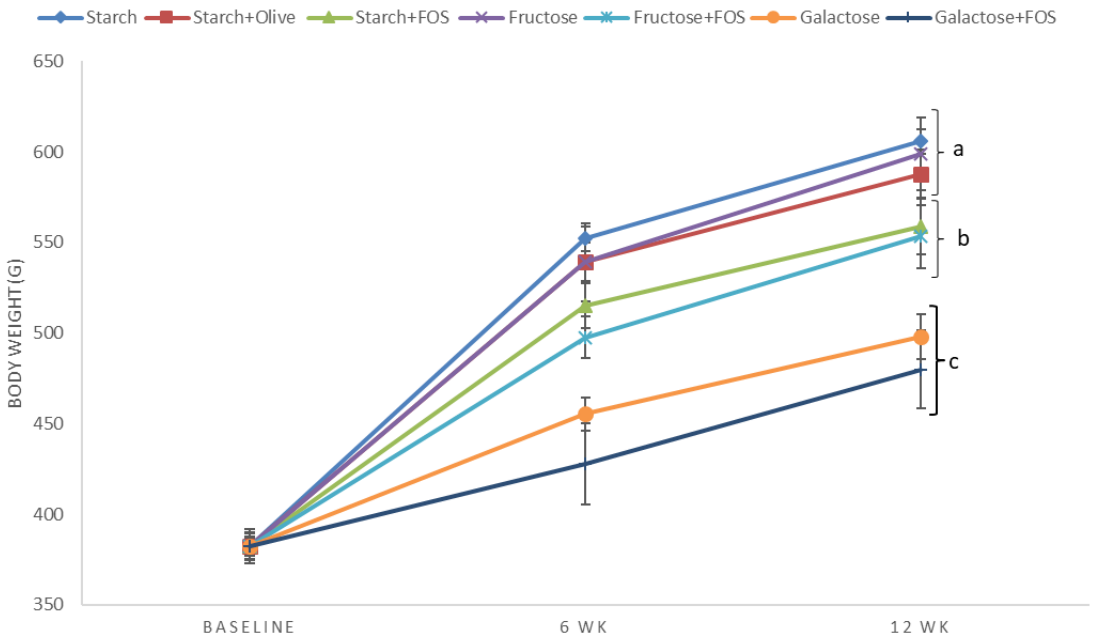

Figure 11. Effects of high fructose or galactose intake on body weight at baseline, six weeks, and 12 weeks. FOS – fructooligosaccharides. Starch = control, starch+FOS = FOS control, starch+olive = negative control. Data are expressed as least squares (LS) mean ± SEM for six rats, assessed by one-way ANOVA followed by Tukey's post hoc test. LS means with different superscripts (lowercase letters) are significantly different (P<0.05)

After 12 weeks of the intervention, rats fed the galactose and galactose+FOS diets had lower body weight than those fed with other diets (**Figure 11**). These results were consistent with previous findings that rats fed a galactose diet failed to grow normally and had lower body weight than the control group [240]. A high dose of galactose also results in galactosemia, due to inefficient uptake of galactose in the liver [194], and galactosuria, which increases secretion of galactose into the urine [241]. Clinical symptoms such as polyuria and opacity of the lens in the eyes were observed in rats fed with these two diets, confirming that 50% galactose has a toxic effect on rats. Accumulation of galactose in the circulation increased galactitol production and caused cataracts in the rats [242].

5.3.2 Effects on blood glucose and insulin

Regulation of blood glucose involves four important organs, the small intestine, pancreas, liver, and muscles. Overall, no differences were observed in blood glucose concentration between the intervention groups. Insulin concentration was significantly higher in rats fed the galactose+FOS diet than in rats fed the starch+FOS diet. HOMA-IR was significantly higher in rats fed the fructose diet than all other diets except fructose+FOS diet (**Figure 12**).

Most of the galactose absorbed from the small intestine is converted into glycogen in the liver [(243)]. Higher levels of galactose in the liver could limit conversion of glucose into glycogen and result in hyperglycemia [(244)]. Moreover, replacement of glucose or fructose by galactose under isocaloric conditions does not alter blood sugar concentration and has been shown to improve hepatic insulin sensitivity [(245)]. Among other simple sugars, fructose has a minimal effect on blood glucose due to slow rate of absorption [(246)]. Fructose also has a low insulinemic response and has been recommended as a sweetener for diabetic individuals [(21)].

Fructose and galactose diet did not have any effects on blood glucose and insulin concentrations, which is in line with previous findings [(21, 247)]. Instead, the galactose with added FOS diet significantly increased insulin concentration, which might be due to expression of incretins, including glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide-1 (GLP-1). GLP-1 binds to the receptors on pancreatic β-cells and induces production and release of insulin, resulting in enhanced insulin concentration [(248, 249)]. Galactose might stimulate incretin secretion, leading to increased insulin release into the circulation [(248)]. Moreover, SFCAs, the main products of fermentation of dietary fiber types such as FOS, have been shown to stimulate excretion of GLP-1 from intestinal L-cells [(249)].

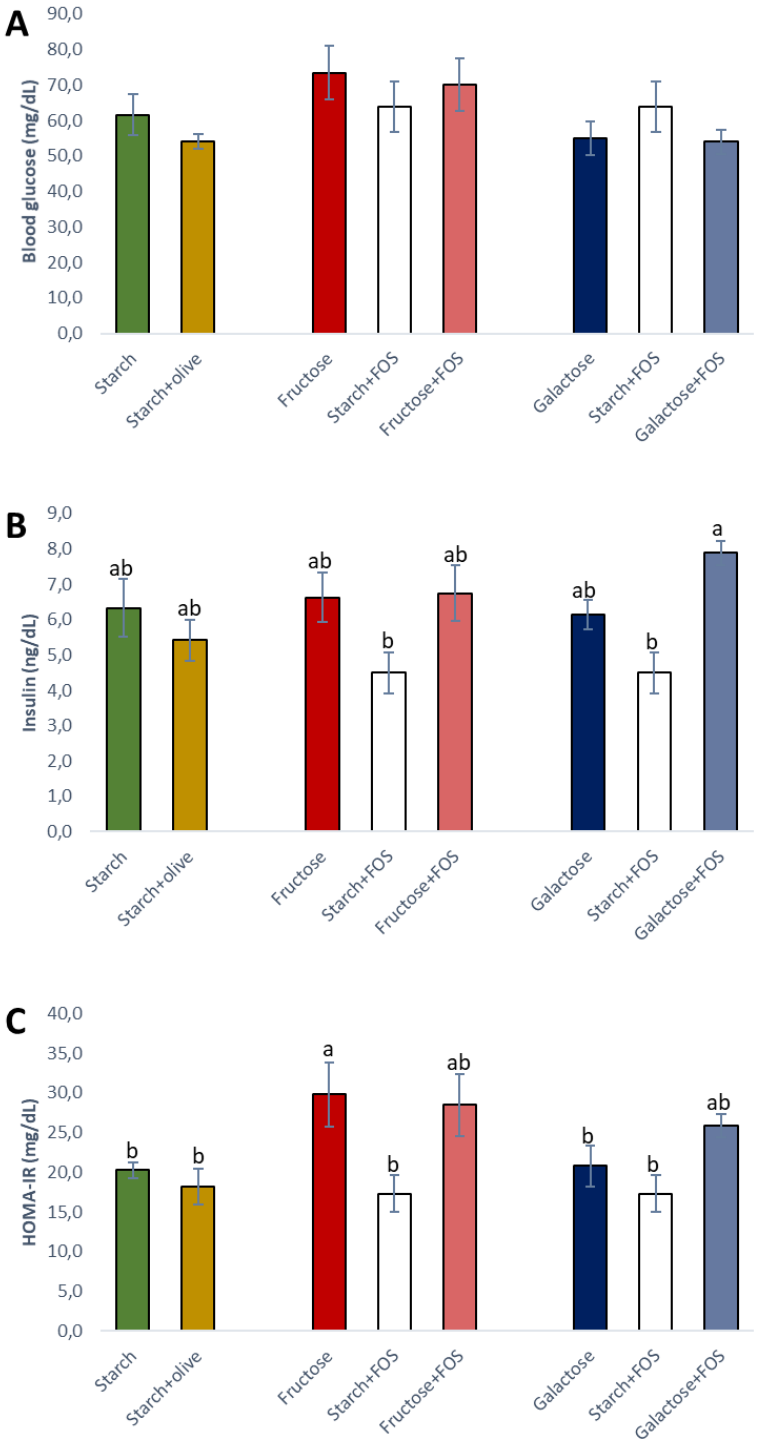

Figure 12. Effects of high fructose intake and high galactose intake on the metabolic factors: **A)** glucose, **B**) insulin, and **C**) Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) in rats after 12 weeks. FOS – fructooligosaccharides. Starch = control, starch+FOS = FOS control, starch+olive = negative control. Data presented as least squares (LS) mean ± SEM for six rats, assessed by one-way ANOVA followed by Tukey's post hoc test. LS means with different superscripts (lowercase letters) are significantly different (P<0.05).

5.3.3 Effects on blood lipids

In general, high intake of sugars has been shown to increase total cholesterol, LDL, and triglycerides [250]. In **Paper IV**, total cholesterol and HDL concentrations were significantly higher in rats fed the fructose, galactose, and galactose+FOS diets compared to rats fed starch+FOS diet. LDL/VLDL cholesterol was significantly higher in rats fed the galactose and fructose+FOS diets than in rats on the starch+FOS (FOS control) diet (**Figure 13**). High intake of fructose has been shown to elevate serum total cholesterol more than sucrose and glucose [251]. A diet high in D-galactose for seven weeks has been found to increase total cholesterol concentration in mice [252]. Overall, no clear effects of added FOS on total cholesterol and HDL concentrations were observed in rats fed the fructose+FOS and galactose+FOS diets compared to rats fed diets without FOS.

Imbalance in circulating atherogenic lipoproteins, including LDL and VLDL/HDL ratio, has been linked with initiation of endothelial dysfunction, which is closely related to the oxidative stress and inflammation that play important roles in generation of atherosclerosis [253]. LDL is the most atherogenic lipoprotein and lowering the LDL concentration through specifically targeted diet and drug therapy could decrease the CHD risk [254]. The finding that higher fructose and galactose led to increased LDL/VLDL concentration is in line with results in previous studies [253]. However, in **Paper IV** there was no difference in rats treated with high fructose, unless it was with added FOS.

Many studies have reported that consumption of diets containing high fructose significantly increases fasting triglycerides [19, 183, 187, 251]. This is explained by fructose metabolism producing a large amount of acetyl-CoA, which plays a central role in *de novo* lipogenesis [187]. The lipogenic characteristic of fructose has become the main concern that fructose may induce hypertriglyceridemia and dyslipidemia [19, 181]. However, no significant differences in triglycerides concentrations across diet groups were found in the present study (**Figure 13B**).

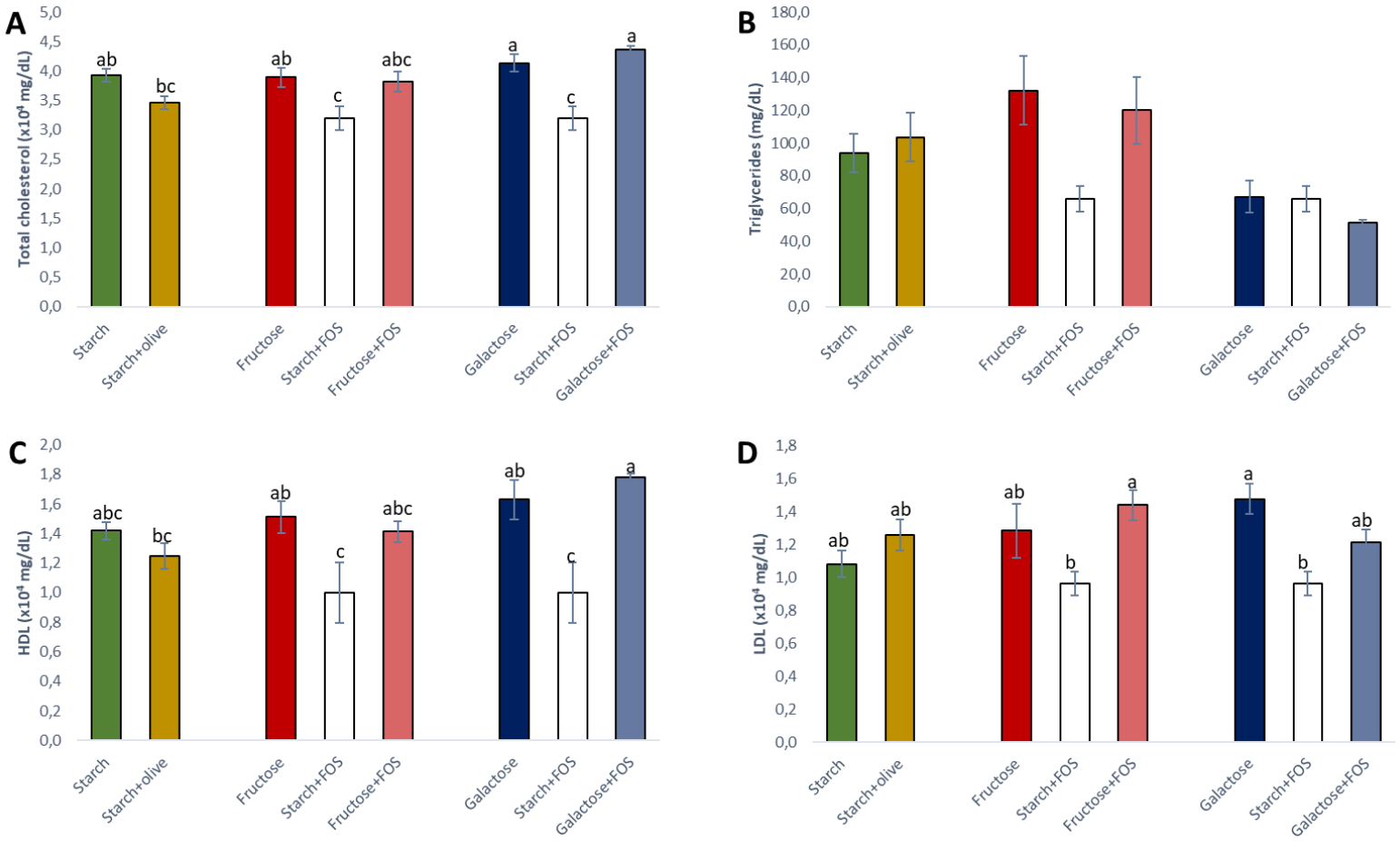

Figure 13. Effects of high fructose intake and high galactose intake on the metabolic factors: **A**) total cholesterol, **B**) high-density lipoprotein (HDL), **C**) low-density lipoprotein/very low-density lipoprotein (LDL/VLDL), and **D**) triglycerides in rats after 12 weeks. FOS - fructooligosaccharides. Starch = control, starch+FOS = FOS control, starch+olive = negative control. Data presented are least squares (LS) mean ± SEM for six rats, assessed by one-way ANOVA followed by Tukey's post hoc test. LS means with different superscript lowercase letters are significantly different, $P<0.05$.

5.3.4 Effects on inflammatory biomarkers

Although high fructose and high galactose were expected to provoke inflammatory markers, the results did not support this. There was no effect of any of the groups compared with the control on CRP, IL-6, TNF-α, and IL-1β concentrations after 12 weeks of the intervention (**Paper IV**). All intervention diets contained high amounts of n-6 PUFA from safflower oil as part of the fat content, as it is suggested to trigger low-grade inflammation [255, 256]. However, no difference in inflammatory biomarkers was observed after the 12-week intervention, suggesting that inflammation was not induced by addition of n-6 PUFA under the conditions in **Paper IV**.

5.3.5 Effects on advanced glycation end products

Two biomarkers of AGEs were measured, N_{ε}-carboxymethyl-lysine (CML) and pentosidine. CML and pentosidine concentrations were significantly higher in rats fed the galactose diet than the in rats on a starch (control), starch+FOS (FOS control), or fructose+FOS diet (**Figure 14**). CML is the most abundant AGE found in different foods (exogenous AGEs) and also in human tissues, and has been widely studied for its roles in metabolic health and age-related diseases [197, 257]. This is in line with previous findings that higher intake of D-galactose induces production of AGEs [178, 258].

Pentosidine is a cross-linked compound with lysine and arginine residues linked to a pentose. A significant higher of lysine concentration was observed in rats fed the galactose+FOS than all other diets. Plasma pentosidine is elevated in inflamed individuals, such as end-stage renal disease patients, and is strongly associated with increased hsCRP and other inflammatory biomarkers, including IL-6, VCAM-1, and ICAM-1 concentrations [259]. Other lysine-rich proteins involved in glycation through lysine residues are hemoglobin, collagen, human serum albumin, HDL and LDL, CML, immunoglobulin G (IgG), immunoglobulin M (IgM), immunoglobulin A (IgA), and histones [260]. However, preferable sites of glycation *in vivo* have been suggested in hemoglobin and serum albumin lysine residues in humans [197, 261].

In general, both CML and pentosidine concentrations were reduced after fructose or galactose diets with added FOS, respectively.

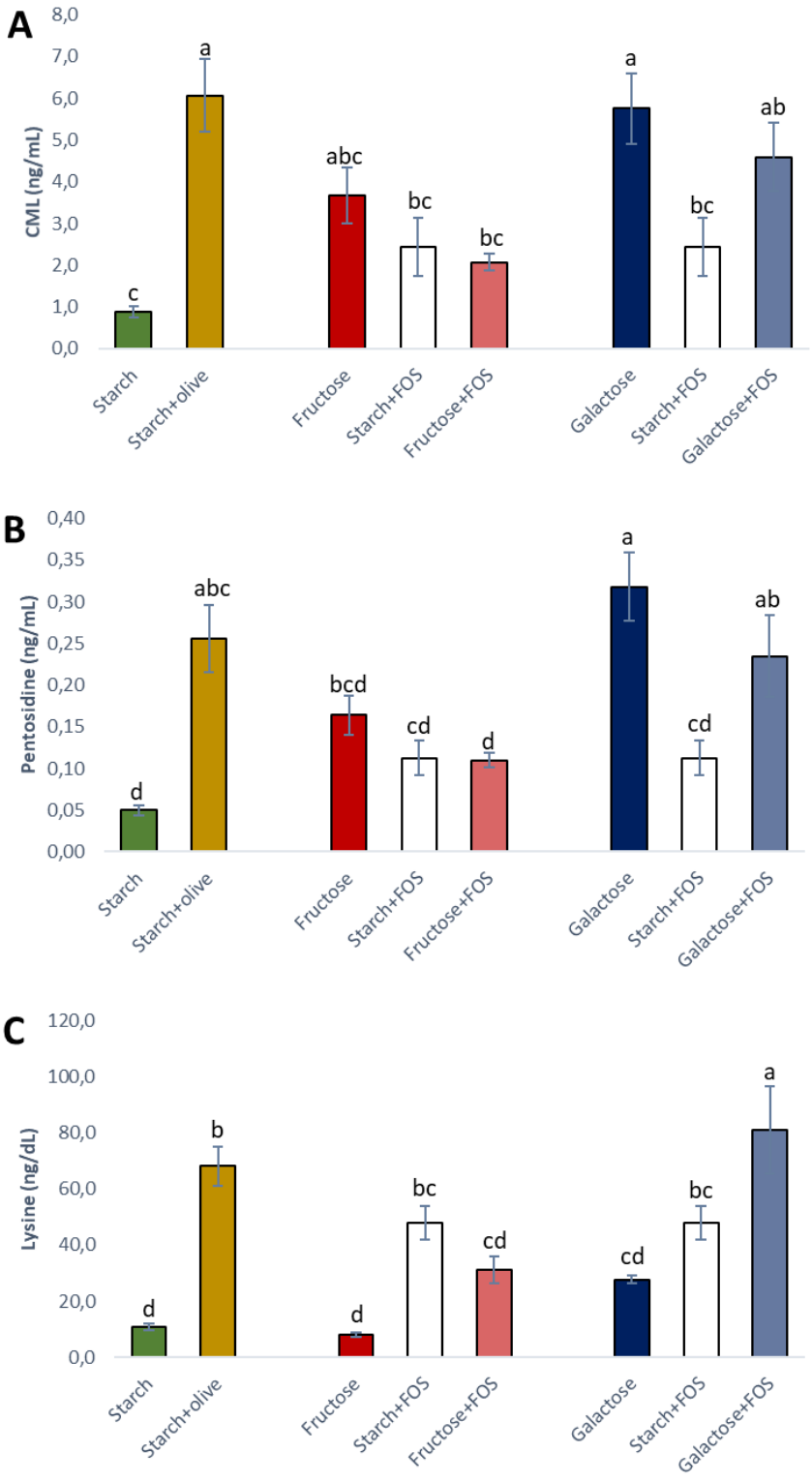

Figure 14. Effects of high fructose intake and high galactose intake on the advanced glycation end products (AGEs): **A)** nε-(carboxymethyl)lysine (CML), **B**) pentosidine, and **C**) lysine in rats after 12 weeks. FOS – fructooligosaccharides. Starch = control, starch+FOS = FOS control, starch+olive = negative control. Data presented are least squares (LS) mean ± SEM for six rats, assessed by one-way ANOVA followed by Tukey's post hoc test. LS means with different superscripts (lowercase letters) are significantly different (P<0.05).

5.3.6 Effects on gut permeability markers

The integrity of the intestinal barrier plays a key role in protecting the body against influx of microbiota-derived products [262]. Increased gut permeability is associated with gut-derived protein fragments, lipopolysaccharides (LPS), in the circulation and dysbiosis or imbalance in the microbiota due to dietary and lifestyle habits [30, 263]. Zonulin and LPS concentrations were measured as biomarkers to determine gut permeability in **Paper IV**.

Zonulin, or zonula occludens toxin (Zot), is a protein that regulates the intestinal tight junction between enterocytes in the gut. Interaction between antigen or fragments of antigen and CXCR3 receptor develops a cascade of responses [264]. MyD88-dependent signaling causes stimulation of zonulin creation, and then zonulin leaves the cells and activates some receptors on enterocyte surfaces, specifically PAR_2 and EGFr. The result of this interaction is decoupling of tight junction protein, which allows the junctions to open, followed by movement of antigen into the circulation [264]. Under pathophysiological conditions, zonulin is stimulated and causes activation of tight junctions, followed by leaky gut barrier and expression of endotoxin in circulation [264].

In **Paper IV,** zonulin concentration was significantly higher in rats fed the galactose+FOS diet than the fructose and starch+olive (negative control) diets. No clear effects of high fructose or high galactose diets on zonulin or endotoxin concentrations were found. However, diets with added FOS appeared to have higher zonulin concentration after 12 wk of intervention compared to diets without FOS (**Figure 15**).

Overall, the results in **Paper IV** suggest that type of diet affect several metabolic factors and gut integrity. A diet high in galactose showed more pronounced adverse effects on insulin, blood lipids, CML, pentosidine, and lysine concentrations than a diet containing high fructose. However, the effects may to some extent have been influenced by the toxic effects of galactose at the very high dose tested.

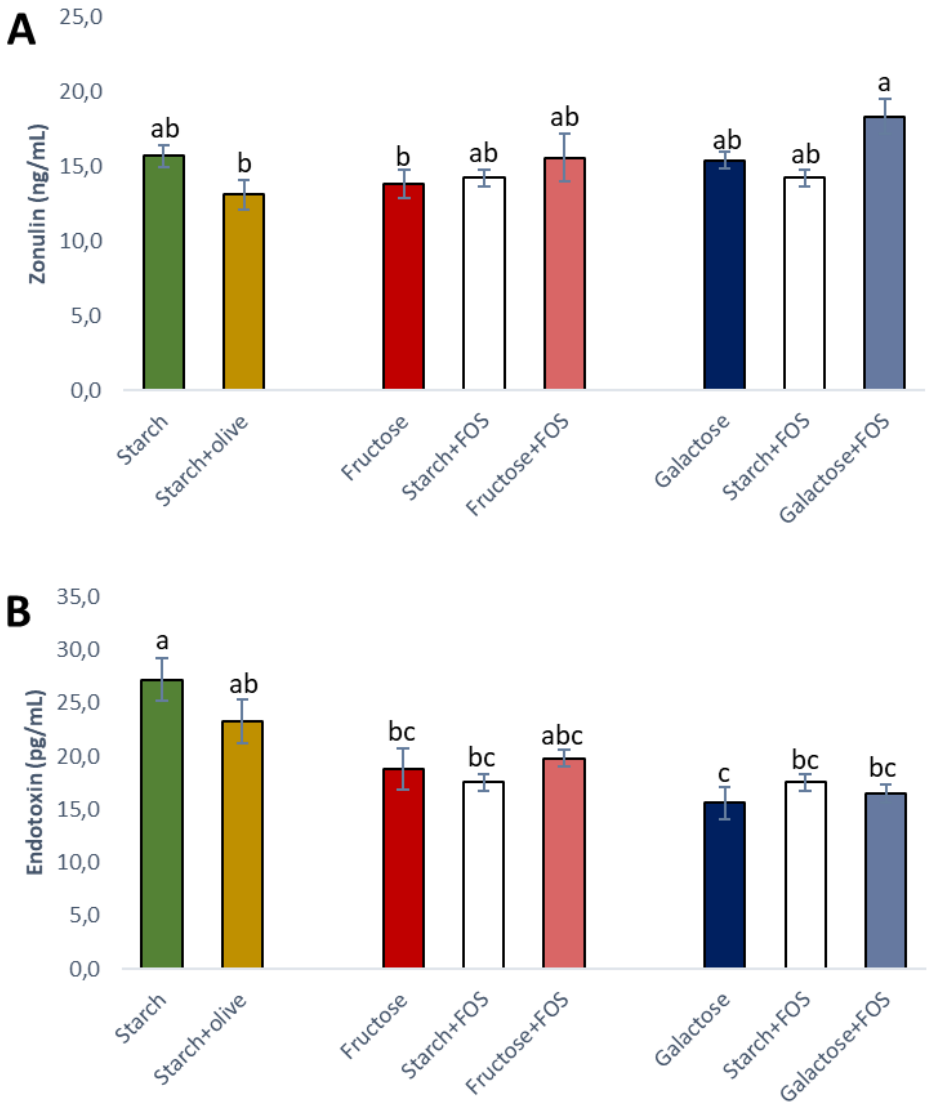

Figure 15. Effects of high fructose intake and high galactose intake on the gut permeability markers: **A**) zonulin and **B**) endotoxin in rats after 12 weeks. FOS – fructooligosaccharides. Starch = control, starch+FOS = FOS control, starch+olive = negative control. Data presented are least squares (LS) mean ± SEM for six rats, assessed by one-way ANOVA followed by Tukey's post hoc test. LS means with different superscripts (lowercase letters) are significantly different ($P<0.05$).

5.3.7 Effects on gut microbiota richness and diversity

Alpha diversity, a measure of microbiota diversity and richness within intestinal samples, was measured in **Paper V**. Significantly lower microbiota richness was found in rats fed the starch+FOS (FOS control) diet compared to all other groups, whereas rats fed the starch+olive (negative control) showed higher microbiota richness. Similarly, significantly lower microbiota diversity was observed in rats fed the starch+FOS (FOS control) diet than all other diets (**Figure 16**).

Overall, the lower microbiota richness and diversity observed in rats fed the starch+FOS (FOS control) diet might be due to inhibition of growth of opportunistic pathogens by prebiotics such as FOS [265, 266].

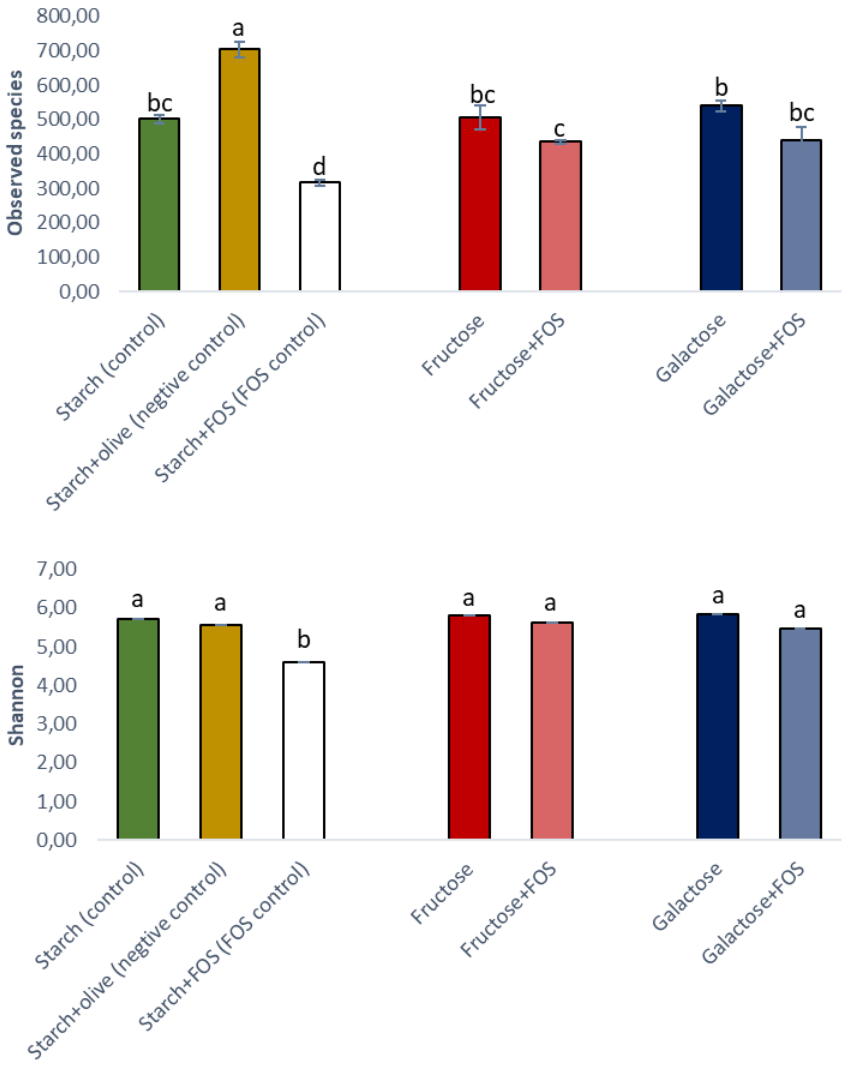

Figure 16. Effects of high fructose intake and high galactose intake on the alpha diversity parameters: **A**) species richness and **B**) Shannon index in the gut of rats after 12 weeks. FOS – fructooligosaccharides. Starch = control, starch+FOS = FOS control, starch+olive = negative control. Data presented are least squares (LS) mean ± SEM for six rats, assessed by one-way ANOVA followed by Tukey's post hoc test. LS means with different superscript lowercase letters are significantly different (P<0.05).

5.3.8 Effects on relative abundance of gut microbiota composition

Beta diversity was analyzed to measure similarity or difference between the treatments. Overall, 22 phyla with more than 300 genera were identified in the samples from the different diets. **Paper V** focused on the relative abundance of seven major microbiota phyla in the large intestine: Firmicutes, Bacteroidetes, Proteobacteria, Cyanobacteria, Actinobacteria, Verrucomicrobia, and Tenericutes (**Figure 17**). These seven phyla were further analyzed to assess the effect of diets and their associations with several selected biomarkers from **Paper IV**.

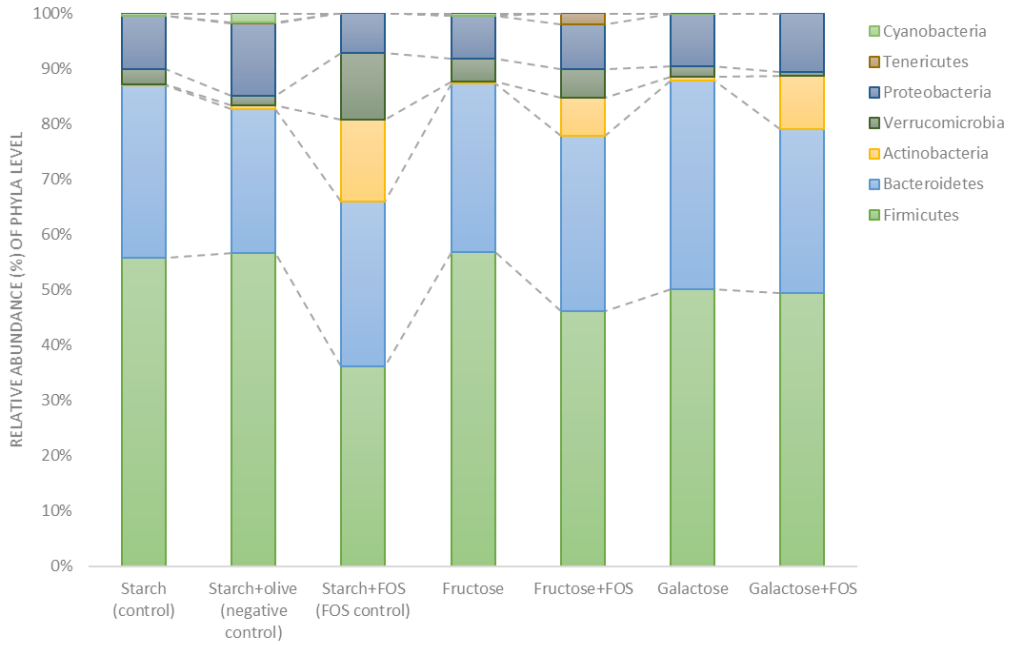

Figure 17. Relative abundance (%) of gut microbiota at phylum level after treatment with high fructose or high galactose, with or without fructooligosaccharides (FOS), in rats after 12 weeks.

The results revealed a significant effect of different diets on changes of gut microbiota composition of Actinobacteria, Verrucomicrobia, Tenericutes, and Cyanobacteria composition. No difference was observed for the phylum Firmicutes, Bacteroidetes, and Proteobacteria.

Ingestion of high fructose saturates its absorption in the small intestine and results in a fraction of fructose reaching the large intestine [267]. In the large intestine, fructose undergoes fermentation by gut microbes and may cause abdominal discomfort such as flatulence, pain, and diarrhea [267, 268]. In **Paper V**, very limited effects of high fructose or high galactose intake on gut microbiota changes at phylum level were observed. However, as expected, the rats fed a diet containing FOS showed increased relative abundance of Actinobacteria. Therefore, changes in the gut microbiota were investigated at the genus level (**Figure 18**).

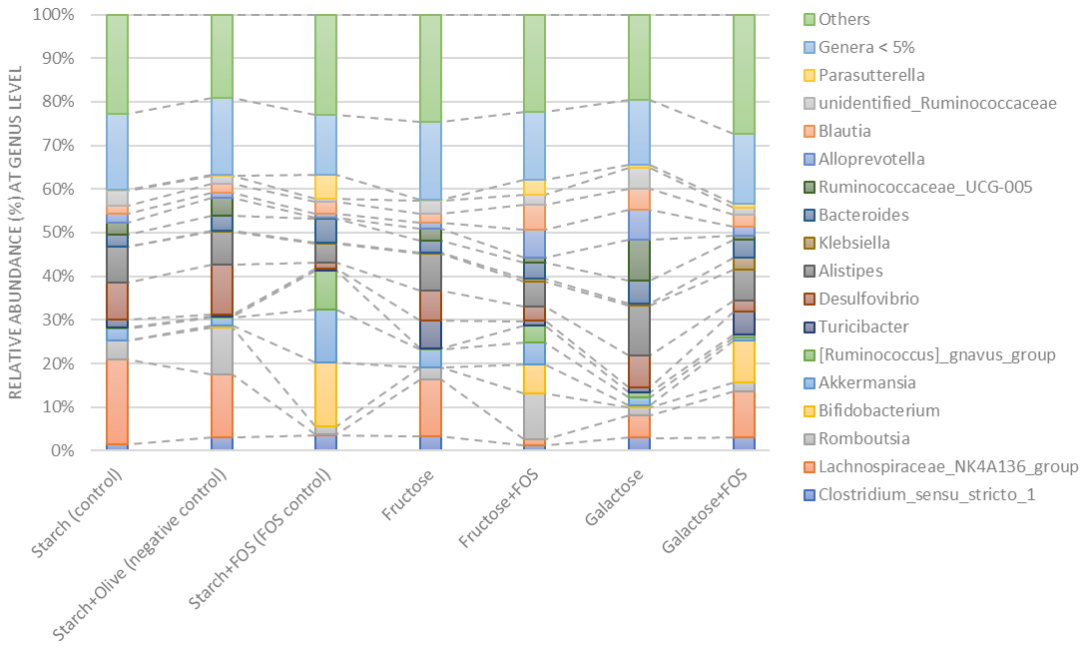

Figure 18. Relative abundance (%) of gut microbiota at genus level (above cut-off level 5.0%) after treatment with high fructose or high galactose, with or without fructooligosaccharides (FOS), in rats after 12 weeks.

Overall, more than 300 genera from 22 phyla were identified. A total of 16 genera with relative abundance >5.0% were determined. In brief, a significant effect of diet was observed for the genera: *Lachnospiraceae_NK4A136_group*, *Bifidobacterium*, *Akkermansia*, *[Ruminococcus]_gnavus_group*, *Desulfovibrio*, *Klebsiella*, *Ruminococcaceae_UCG-005*, *Alloprevotella*, *unidentified_Ruminococcaceae*, *and Parasutterella*.

The effect of FOS on gut microbiota composition was clearly apparent at phylum level. Higher relative abundance of the phylum Actinobacteria was dominated by the genus *Bifidobacterium* (**Figure 18**). Many potential beneficial health effects of FOS have been studied, such as lower cholesterol and triglyceride concentrations and improved absorption of minerals [150, 269, 270]. FOS is thoroughly fermented by gut microbiota and metabolized into SCFAs, gases including CO_2 and hydrogen, and lactate [28, 159, 160]. The main components of SCFAs, acetate, propionate, and butyrate, can acidify the colon and support growth of *Bifidobacterium* [266].

5.3.9 Association between gut microbiota and selected metabolic, inflammatory, and gut permeability markers

Seven gut microbiota phyla were associated with several selected metabolic factors (blood glucose, insulin, HOMA-IR), inflammatory biomarkers (CRP, IL-6, IL-1β, TNF-α), AGEs (CML, pentosidine, lysine), and gut permeability markers (endotoxin and zonulin).

Correlations between gut microbiota phyla and selected biomarkers are shown in a correlation matrix in **Figure 19**. Overall, Firmicutes and Cyanobacteria were positively associated with body weight (r=0.54, $P<0.001$ and r=0.51, $P<0.001$, respectively). In contrast, Bacteroidetes and Actinobacteria showed an inverse association with body weight (r=-0.37, $P<0.05$ and r=-0.45, $P<0.01$, respectively). Moreover, Actinobacteria was also significantly associated with lysine (r=0.52, $P<0.01$) but inversely associated with endotoxin (r=-0.38, $P<0.05$). Verrucomicrobia was inversely associated with CML (r=-0.39, $P<0.05$) and pentosidine (r=-0.38, $P<0.05$) concentrations. Cyanobacteria was positively associated with endotoxin concentration (r=0.43, $P<0.01$). Proteobacteria and Tenericutes did not show an association with any of the biomarkers studied.

Associations between specific gut microbiota and clinical measures could provide insights into the potential involvement of specific gut microbiota in the host metabolism and provide hints about underlying mechanisms [271, 272]. Moreover, the findings could indicate targets for prevention and therapeutics of pathophysiological conditions.

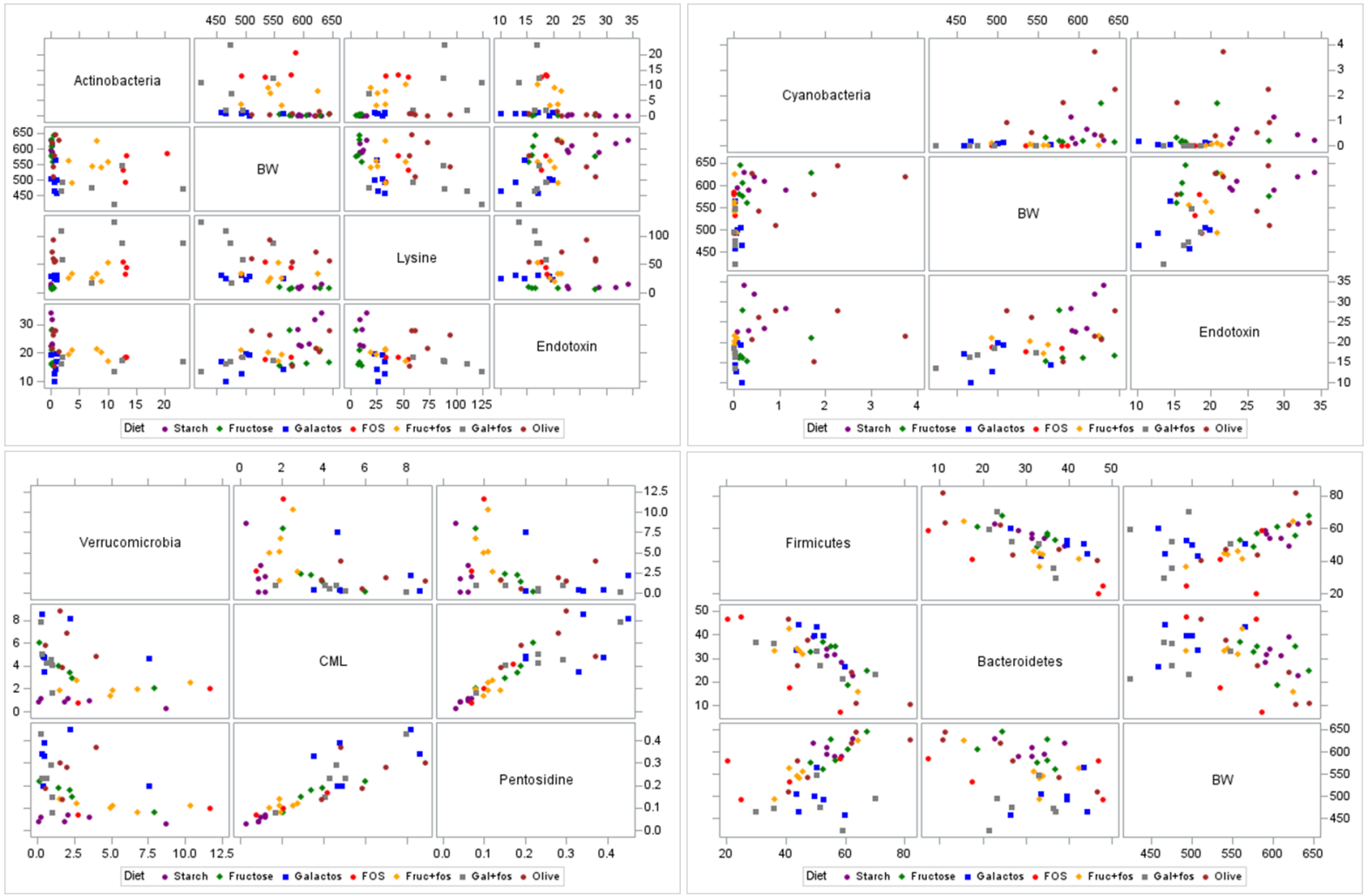

Figure 19. Correlation matrix of the phyla Firmicutes, Bacteroidetes, Actinobacteria, Cyanobacteria, and Verrucomicrobia versus metabolic factors and gut permeability markers in rats fed a high fructose or galactose diet, with and without added fructooligosaccharides.
The x-axis and y-axis indicate relative abundance (%) of microbiota or concentration of metabolic factors.

5.3.10 Methodological considerations

In this thesis, three different study designs (human observational study, human experimental/intervention study, and animal experimental study) were used to address the main research objectives. All these types of study have their strengths and limitations.

*Human observational studies (**Papers I** and **II**)*

A cross-sectional study design was used in **Papers I** and **II**. The strength of the studies is that the study participants, both men and women, represented a general population, in contrast to studies based on some selected groups of a population. Another strength is that various tools were used to reflect consumption of whole grain rye and wheat, *i.e.*, self-reported intake in food frequency questionnaires and AR concentrations in plasma and adipose tissue. The studies also have some limitations. For example, recall bias may have been introduced to some extent when the study participants were asked to recall and self-report their food intake in general, and intake of whole grain products in particular. It is difficult for people to remember the exact frequency of consumption of specific foods, and therefore random misclassification may occur. However, repeated assessments of whole grain foods were used to decrease random error in reporting and to better reflect long-term diet. Studies have shown that self-reports of diet may also systematically differ depending on characteristics of the study participants, such as age, obesity, and education level [273]. This may lead to systematic misclassification of whole grain intake. However, all participants in the two studies were elderly and within a relatively small range of age and BMI. Confounding could also have affected the true association between whole grain intake and the studied outcomes, but potential confounding factors were controlled for in the statistical analyses by including multivariate models [274]. The adjusted confounders for **Papers I** and **II** are listed in **Table 4**. The relatively small sample size may also be a limitation of these two studies [275]. However, although the sample size was small, the study was still able to find associations between whole grain intake estimated from FFQs and based on measurements of ARs in plasma with selected biomarkers.

*Human experimental study (**Paper III**)*

The human intervention study presented in **Paper III** was an exploratory study of whole grain intake effects on inflammation biomarkers, since the primary aim was to investigate the effects of WG/bran rye on prostate specific antigen (PSA) after six weeks in men with low-grade prostate cancer [203]. Due to the exploratory nature of the study, no power calculations were performed, but it is obvious that the sample size (17 men) was small and the results should therefore be interpreted with caution. Despite the small sample size, suggestions of effects of

WG/bran rye compared with refined wheat products on several inflammatory biomarkers were found. These need to be confirmed in future well-designed intervention studies with appropriate statistical power.

*Experimental animal studies (**Papers IV** and **V**)*

In the intervention studies in rats, the doses of fructose and galactose used in the experimental diets were high, in order to evoke low-grade inflammation and to increase the likelihood that some sugars would reach the colon. In those studies, clinical toxic symptoms were observed in rats fed diets containing high galactose concentrations, which required an immediate change in the design of the study. The results should therefore be interpreted with caution. Moreover, results from rats may not necessarily be directly transferrable to humans [276]. In addition, the gut microbiota in rats is different from that in humans. However, an animal model allowed an intervention with high doses of the sugars that would be impossible to conduct in humans, and was able to provide more information and a wider understanding.

6 GENERAL DISCUSSION

It is evident that carbohydrate quality in the diet has major implications for cardiometabolic diseases, through modulation of risk factors such as low-grade inflammation, insulin resistance, and dyslipidemia [9, 10]. Evaluations of carbohydrate quality through assessment of type of whole grain, source of dietary fiber, sugars, and glycemic index have shown important effects on health outcomes [3, 9, 16]. However, the role of specific whole grains on cardiometabolic risk factors is still largely unknown. Moreover, studies on the role of whole grain intake in low-grade inflammation and potential underlying mechanisms are limited. Furthermore, while studies have suggested that some of the adverse effects of sugars on metabolic risk factors could be mediated by gut microbiota, direct evidence is still lacking (30, 31). The studies presented in this thesis addressed some of the knowledge gaps on carbohydrate quality and its effect on health.

Inflammation is a hallmark of cardiometabolic diseases and it is therefore essential to determine the potential of diet to modulate this risk factor [125]. Several mechanisms for a direct and indirect action of whole grains in inflammation have been suggested, including effects of phytochemicals, fiber, and metabolites produced by gut microbiota [12, 132, 277, 278]. In both intervention and observational studies, the effects of whole grains on inflammatory biomarkers have been more pronounced in unhealthy than healthy subjects [279, 280].

In the studies included in this thesis, the emphasis was on the role of whole grain rye and wheat, the main cereals consumed in Sweden and many Northern European countries, in modulation of inflammatory, endothelial function, and CVD risk-related biomarkers. Comprehensive strategies were used to estimate whole grain rye and wheat intake, relying on both FFQs and dietary biomarkers in plasma and adipose tissue (**Paper I**). The results revealed a positive association between whole grain rye and wheat and cathepsin S, and a negative association between total alkylresorcinols and C17:0/C21 ratio in adipose tissue and endostatin. This suggests a potential effect of whole grain rye and wheat in modulation of cathepsin S and endostatin. Further studies are required under intervention conditions to confirm these findings. In the assessment of alkylresorcinols in adipose tissue as a biomarker of long-term while grain intake (**Paper II**), the same subjects and data as in **Paper I** were used. It was found that alkylresorcinol concentration in adipose tissue was significantly correlated with whole grain rye, but a poor correlation was observed for whole grain wheat. The inconsistent results (**Paper I**) and weak correlation obtained (**Paper II**) are most likely due to poor estimation of whole grain wheat intake from FFQs. Food frequency questionnaires with many specific cereal food items could be an alternative for improvement [281].

Comparisons of the ability of whole grain rye bran and refined wheat to modulate inflammatory biomarkers and endothelial function in men with low-grade prostate cancer showed that consumption of whole grain rye bran had a pronounced effect in reducing several biomarkers,

including TNF-R2, endostatin, and e-selectin concentrations, compared with refined wheat (**Paper III**). Refined wheat contains lower amounts of phytonutrients than whole grain rye bran, due to removal during processing of bran and germ parts, which contain the main beneficial components [277]. These findings in this thesis suggest that the effects of whole grain rye bran on some biomarkers may derive from phytochemical components and dietary fiber content in the product. Although this exploratory study was small and requires confirmation in larger settings, the results support the general dietary guideline of replacing refined grain with whole grains when cereals are consumed.

The adverse effects of high fructose or high galactose intake on metabolic factors were unclear, and the net effects were complex to determine in the experimental animal setting. Although it was difficult to find consistent adverse effects of these sugars, high galactose tended to increase advanced glycation end product concentrations over time. A diet containing galactose showed greater effects than fructose, but the results might be confounded by clinical symptoms due to the high dose (**Paper IV**). The effect of fructooligosaccharides in alleviating potential effects of these sugars was less pronounced. No coherent effects were found on gut microbiota after treatment with intervention diets, except for increased abundance of the genus *Bifidobacterium* after consumption of fructooligosaccharides (**Paper V**). Fermentation of fructooligosaccharides by gut microbiota and production of short-chain fatty acids might play a role in absorption of monosaccharides and gut microbiota activity [141, 160, 282], and this issue requires further investigation. In addition, intestinal alkaline phosphatase in the gastrointestinal tract may protective effects against systemic and intestinal inflammation, by inhibiting activation of NF-κB and preventing expression of pro-inflammatory cytokines [283]. This also needs further study.

In summary, the work presented in this thesis showed that whole grain rye and wheat may have potential effects in regulation of low-grade inflammation, confirming previous findings, and that alkylresorcinol concentration in adipose tissue has potential as a long-term biomarker of whole grain rye intake. High fructose and high galactose diets showed minor effects on metabolic factors and gut microbiota composition. Further studies are needed to confirm these findings.

7 MAIN FINDINGS AND CONCLUSIONS

- Whole grain rye and wheat consumption was positively associated with cathepsin S, whereas total alkylresorcinol and C17:0/C21:0 ratio in plasma were negatively associated with endostatin concentrations.

- Total alkylresorcinol concentration in adipose tissue was associated with long-term whole grain rye consumption and appears to be a promising biomarker of long-term whole grain rye consumption, after adjustment for sex. Total alkylresorcinol concentration in adipose tissue was weakly correlated with whole grain wheat consumption.

- Consumption of whole grain rye/bran products significantly lowered endostatin, e-selectin, and TNF-R2 concentrations in men with low-grade prostate cancer, in parallel with reductions in prostate-specific antigen, compared with refined wheat products with added cellulose.

- High intake of fructose or galactose, with or without added fructooligosaccharides, affected several metabolic factors and gut permeability markers to some degree compared with control diets, but did not cause differences in inflammatory markers. High-galactose diets, with or without added FOS, had greater adverse effects on metabolic factors than high-fructose diets. There was no clear general benefit of added fructooligosaccharides in the diet as a strategy to mitigate adverse effects of high fructose and high galactose intake.

- Intake of high fructose or high galactose did not cause pronounced effects on microbiota composition, but some effects of the diets were found at certain time points. As expected, added fructooligosaccharides significantly increased the abundance of the genus *Bifidobacterium*.

- Gut microbiota (at phyla level) was correlated with several selected metabolic factors, advanced glycation end products, and gut permeability markers.

8 FUTURE RESEARCH

- The effects of whole grain rye consumption on low-grade inflammation and endothelial dysfunction should be further investigated in a larger study population and in intervention settings.

- Total alkylresorcinol concentration in adipose tissue as a long-term biomarker of whole grain wheat and rye intake should be further evaluated in populations with lower whole grain intake.

- A lower dose of galactose than in the present study should be tested in future rat studies, to avoid clinical symptoms that could affect the results.

- Different doses of fructose should be tested in future, to determine the dose that may potentially modulate low-grade inflammation.

- Histological studies on different animal organs should be carried out to determine potential effects of high-fructose and high-galactose diets.

- The effect of intestinal alkaline phosphatase should be further investigated, to determine its role in gut integrity, inflammation, and gut microbiota composition in rats treated with high-fructose and high-galactose diets.

- The role of gut microbiota-derived metabolites, such as short chain fatty acids, in absorption of monosaccharides in the small instestine should be investigated.

- Gut microbiota should be further investigated at species level in animals on diets containing high fructose and high galactose.

9 ACKNOWLEDGEMENTS

All the work in this thesis was performed at the Department of Food Science (now Department of Molecular Sciences), Swedish University of Agricultural Sciences, Uppsala, Sweden; the Department of Biology and Biological Engineering, Food and Nutrition Science Division, Chalmers University of Technology, Gothenburg, Sweden; the Unit for Cardiovascular and Nutritional Epidemiology, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden; and the Institute of Biological Chemistry and Nutrition, University of Hohenheim, Stuttgart, Germany. It involved many years of hard work, tears, and sweat, and the help of various excellent academic advisors with different backgrounds and specialties. I would like to express the greatest level of gratitude for the priceless experience and knowledge I gained, particularly to:

Ministry of Higher Education Malaysia & Universiti Malaysia Pahang: Special thanks and appreciation for the financial support throughout my studies in Sweden.

Rikard Landberg: Thank you for your continuous support of my PhD study and related research, for your patience, motivation, and immense knowledge. Your guidance helped me during all time of research and writing this thesis. I could not imagine having a better supervisor for my PhD study.

Alicja Wolk: Thank you for the great opportunities and all support and help that you have given me. Much of this work would not been possible without your guidance and endless support. I am grateful to have had you as one of my supervisors.

Galia Zamaratskaia: My sincere appreciation to you for your supervision and encouragement. Thank you for all the helpful discussions throughout the project, always coming up with fantastic ideas. It was a privilege to work with you.

Chalmers and SLU Colleagues: Special thanks to my 'lovely neighbor' Bita, Nuria '*PIC*' and Gabriel. Loved every single day with you here at the division, with all wonderful stories and silly ideas. I'm leaving – and starting to miss you guys! Izabela, Nafisa, Joao, Therese ~ awesome officemates! Kia, Lin, Jing, Ken, Huaxing, Susanne, Elise, Serafina, Rikard F, Karin, Marie A (mentor), and all from FNS ~, CMSI; Otto, Jacob, Souad (ex-CMSI).

University of Hohenheim Colleagues: Jan Frank, thank you for the great opportunities in Stuttgart and for all help with the rats. Thank you to Nadine and Johanita for supporting me during my time in Stuttgart and giving me a lot of assistance during my lab work in Stuttgart.

Malaysian and besties in Gothenburg, Uppsala, Stockholm, and around Sweden: Thanks to all friends in Gothenburg and Uppsala for your kindness in accepting me and my family to be part of the big family here in Gothenburg and Uppsala. See you in Malaysia or else!

Special thanks to Mhd Omar Husain (*Ayah*) and Zaiton Mohamad (*Mak*) for being the greatest parents in the world, always praying for my success. Thank you also to my siblings, in-laws, besties from UMP, UM, and else for the support, care, and love they provide. I love you! ☺

Lastly, to my *abang* Mohd Izwan Kamarul Bahar. Thank you for being my superHero! superDad! superChef! To my beautiful twins Nur Dalili and Muhammad Daniyal, thank you for being around and putting a smile on my face all the time! I love you! ☺

10 REFERENCES

1. Srivastava AK. Challenges in the treatment of cardiometabolic syndrome. Indian J Pharmacol. 2012;44(2):155-6.

2. Petersen KS, Flock MR, Richter CK, Mukherjea R, Slavin JL, Kris-Etherton PM. Healthy Dietary Patterns for Preventing Cardiometabolic Disease: The Role of Plant-Based Foods and Animal Products. Curr Dev Nutr. 2017;1(12):cdn.117.001289.

3. Ludwig DS, Hu FB, Tappy L, Brand-Miller J. Dietary carbohydrates: role of quality and quantity in chronic disease. BMJ. 2018;361:k2340.

4. Willett WC, Koplan JP, Nugent R, Dusenbury C, Puska P, Gaziano T. Prevention of Chronic Disease by Means of Diet and Lifestyle Changes. In: T JD, G BJ, R MA, Alleyne G, Claeson M, Evans DB, et al., editors. Disease Control Priorities in Developing Countries. 2nd ed. Washington, D.C.: World Bank; 2006. p. 833-50.

5. Rippe JM. Lifestyle Strategies for Risk Factor Reduction, Prevention, and Treatment of Cardiovascular Disease. Am J Lifestyle Med. 2018;13(2):204-12.

6. Cummings JH, Stephen AM. Carbohydrate terminology and classification. European journal of clinical nutrition. 2007;61(1):S5-S18.

7. Lunn J, Buttriss JL. Carbohydrates and dietary fibre. Nutrition Bulletin. 2007;32(1):21-64.

8. Brouns F. Saccharide Characteristics and Their Potential Health Effects in Perspective. Front Nutr. 2020;7:75-.

9. Blaak EE. Carbohydrate quantity and quality and cardio-metabolic risk. Current opinion in clinical nutrition and metabolic care. 2016;19(4):289-93.

10. Reynolds A, Mann J, Cummings J, Winter N, Mete E, Te Morenga L. Carbohydrate quality and human health: a series of systematic reviews and meta-analyses. The Lancet. 2019;393(10170):434-45.

11. Song X, Bao M, Li D, Li YM. Advanced glycation in d-galactose induced mouse aging model. Mechanisms of Ageing and Development. 1999;108(3):239-51.

12. McRae MP. Health Benefits of Dietary Whole Grains: An Umbrella Review of Meta-analyses. J Chiropr Med. 2017;16(1):10-8.

13. Melanson KJ, Angelopoulos TJ, Nguyen VT, Martini M, Zukley L, Lowndes J, et al. Consumption of whole-grain cereals during weight loss: effects on dietary quality, dietary fiber, magnesium, vitamin B-6, and obesity. Journal of the American Dietetic Association. 2006;106(9):1380-8; quiz 9-90.

14. Tighe P, Duthie G, Vaughan N, Brittenden J, Simpson WG, Duthie S, et al. Effect of increased consumption of whole-grain foods on blood pressure and other cardiovascular risk markers in healthy middle-aged persons: a randomized controlled trial. The American Journal of Clinical Nutrition. 2010;92(4):733-40.

15. Kristensen M, Toubro S, Jensen MG, Ross AB, Riboldi G, Petronio M, et al. Whole grain compared with refined wheat decreases the percentage of body fat following a 12-week, energy-restricted dietary intervention in postmenopausal women. J Nutr. 2012;142(4):710-6.

16. Riccardi G, Costabile G. Carbohydrate quality is key for a healthy and sustainable diet. Nature Reviews Endocrinology. 2019;15(5):257-8.

17. Smith CE, Tucker KL. Health benefits of cereal fibre: a review of clinical trials. Nutr Res Rev. 2011;24(1):118-31.

18. Huang T, Xu M, Lee A, Cho S, Qi L. Consumption of whole grains and cereal fiber and total and cause-specific mortality: prospective analysis of 367,442 individuals. BMC medicine. 2015;13(1):59.

19. Taskinen M-R, Packard CJ, Borén J. Dietary Fructose and the Metabolic Syndrome. Nutrients. 2019;11(9):1987.

20. Laaksonen DE, Toppinen LK, Juntunen KS, Autio K, Liukkonen KH, Poutanen KS, et al. Dietary carbohydrate modification enhances insulin secretion in persons with the metabolic syndrome. Am J Clin Nutr. 2005;82(6):1218-27.

21. Qi X, Tester RF. Fructose, galactose and glucose – In health and disease. Clinical Nutrition ESPEN. 2019;33:18-28.

22. Glushakova O, Kosugi T, Roncal C, Mu W, Heinig M, Cirillo P, et al. Fructose induces the inflammatory molecule ICAM-1 in endothelial cells. Journal of the American Society of Nephrology : JASN. 2008;19(9):1712-20.

23. Marques C. Fructose consumption and its impact on human health: Diet and risk of cardiovascular disease. Revista portuguesa de cardiologia : orgao oficial da Sociedade Portuguesa de Cardiologia = Portuguese journal of cardiology : an official journal of the Portuguese Society of Cardiology. 2017;36(12):943-4.

24. Sadigh-Eteghad S, Majdi A, McCann SK, Mahmoudi J, Vafaee MS, Macleod MR. D-galactose-induced brain ageing model: A systematic review and meta-analysis on cognitive outcomes and oxidative stress indices. PLoS One. 2017;12(8):e0184122-e.

25. Lau C, Toft U, Tetens I, Richelsen B, Jørgensen T, Borch-Johnsen K, et al. Association between dietary glycemic index, glycemic load, and body mass index in the Inter99 study: is underreporting a problem? Am J Clin Nutr. 2006;84(3):641-5.

26. Kang HM, Kim D-J. Total energy intake may be more associated with glycemic control compared to each proportion of macronutrients in the korean diabetic population. Diabetes Metab J. 2012;36(4):300-6.

27. Kyrø C, Tjønneland A. Whole grains and public health. BMJ. 2016;353:i3046.

28. Chassard C, Lacroix C. Carbohydrates and the human gut microbiota. Current opinion in clinical nutrition and metabolic care. 2013;16(4):453-60.

29. Lee Y-K. Effects of diet on gut microbiota profile and the implications for health and disease. Biosci Microbiota Food Health. 2013;32(1):1-12.

30. Mastrocola R, Ferrocino I, Liberto E, Chiazza F, Cento AS, Collotta D, et al. Fructose liquid and solid formulations differently affect gut integrity, microbiota composition and related liver toxicity: a comparative in vivo study. J Nutr Biochem. 2018;55:185-99.

31. Lambertz J, Weiskirchen S, Landert S, Weiskirchen R. Fructose: A Dietary Sugar in Crosstalk with Microbiota Contributing to the Development and Progression of Non-Alcoholic Liver Disease. Front Immunol. 2017;8(1159).

32. Knudsen MD, Kyrø C, Olsen A, Dragsted LO, Skeie G, Lund E, et al. Self-reported whole-grain intake and plasma alkylresorcinol concentrations in combination in relation to the incidence of colorectal cancer. American journal of epidemiology. 2014;179(10):1188-96.

33. Freedman LS, Kipnis V, Schatzkin A, Tasevska N, Potischman N. Can we use biomarkers in combination with self-reports to strengthen the analysis of nutritional epidemiologic studies? Epidemiologic perspectives & innovations : EP+I. 2010;7(1):2.

34. Kirk EP, Klein S. Pathogenesis and pathophysiology of the cardiometabolic syndrome. J Clin Hypertens (Greenwich). 2009;11(12):761-5.

35. Cannon CP. Cardiovascular disease and modifiable cardiometabolic risk factors. Clinical cornerstone. 2007;8(3):11-28.

36. Baygi F, Herttua K, Jensen OC, Djalalinia S, Mahdavi Ghorabi A, Asayesh H, et al. Global prevalence of cardiometabolic risk factors in the military population: a systematic review and meta-analysis. BMC Endocrine Disorders. 2020;20(1):8.

37. Papakonstantinou E, Lambadiari V, Dimitriadis G, Zampelas A. Metabolic syndrome and cardiometabolic risk factors. Current vascular pharmacology. 2013;11(6):858-79.

38. Schmidt MI, Saad MJA, Duncan BB. Subclinical inflammation and obesity, diabetes and related disorders. Drug Discovery Today: Disease Mechanisms. 2005;2(3):307-12.

39. Kim MH, Kim HN, Choi WS. The association between subclinical inflammation and abnormal glucose and lipid metabolisms in normal-weight Korean individuals. Nutrition, Metabolism and Cardiovascular Diseases. 2018;28(11):1106-13.

40. León-Pedroza JI, González-Tapia LA, del Olmo-Gil E, Castellanos-Rodríguez D, Escobedo G, González-Chávez A. [Low-grade systemic inflammation and the development of metabolic diseases: from the molecular evidence to the clinical practice]. Cirugia y cirujanos. 2015;83(6):543-51.

41. Festa A, D'Agostino R, Jr., Howard G, Mykkänen L, Tracy RP, Haffner SM. Chronic subclinical inflammation as part of the insulin resistance syndrome: the Insulin Resistance Atherosclerosis Study (IRAS). Circulation. 2000;102(1):42-7.

42. Willett WC, Koplan JP, Nugent R, Dusenbury C, Puska P, Gaziano TA. Prevention of Chronic Disease by Means of Diet and Lifestyle Changes. In: Jamison DT, Breman JG, Measham AR, Alleyne G, Claeson M, Evans DB, et al., editors. Disease Control Priorities in Developing Countries. Washington (DC)

New York: The International Bank for Reconstruction and Development / The World Bank

Oxford University Press

43. Klein S, Sheard NF, Pi-Sunyer X, Daly A, Wylie-Rosett J, Kulkarni K, et al. Weight management through lifestyle modification for the prevention and management of type 2

diabetes: rationale and strategies. A statement of the American Diabetes Association, the North American Association for the Study of Obesity, and the American Society for Clinical Nutrition. Am J Clin Nutr. 2004;80(2):257-63.

44. Ofei F. Obesity - a preventable disease. Ghana Med J. 2005;39(3):98-101.

45. Kaila B, Raman M. Obesity: a review of pathogenesis and management strategies. Can J Gastroenterol. 2008;22(1):61-8.

46. Hill JO, Wyatt HR, Peters JC. Energy balance and obesity. Circulation. 2012;126(1):126-32.

47. Romieu I, Dossus L, Barquera S, Blottière HM, Franks PW, Gunter M, et al. Energy balance and obesity: what are the main drivers? Cancer Causes Control. 2017;28(3):247-58.

48. Lavie CJ, Milani RV, Ventura HO. Obesity and Cardiovascular Disease: Risk Factor, Paradox, and Impact of Weight Loss. Journal of the American College of Cardiology. 2009;53(21):1925-32.

49. Soriano-Maldonado A, Aparicio VA, Félix-Redondo FJ, Fernández-Bergés D. Severity of obesity and cardiometabolic risk factors in adults: Sex differences and role of physical activity. The HERMEX study. International journal of cardiology. 2016;223:352-9.

50. Han TS, Williams K, Sattar N, Hunt KJ, Lean ME, Haffner SM. Analysis of obesity and hyperinsulinemia in the development of metabolic syndrome: San Antonio Heart Study. Obesity research. 2002;10(9):923-31.

51. Kahn BB, Flier JS. Obesity and insulin resistance. The Journal of Clinical Investigation. 2000;106(4):473-81.

52. Tangvarasittichai S, Pongthaisong S, Meemark S, Tangvarasittichai O. Abdominal Obesity Associated with Elevated Serum Butyrylcholinesterase Activity, Insulin Resistance and Reduced High Density Lipoprotein-Cholesterol Levels. Indian J Clin Biochem. 2015;30(3):275-80.

53. Linton MF, Yancey PG, Davies SS, Jerome WG, Linton EF, Song WL, et al. The Role of Lipids and Lipoproteins in Atherosclerosis. In: Feingold KR, Anawalt B, Boyce A, Chrousos G, de Herder WW, Dungan K, et al., editors. Endotext. South Dartmouth (MA): MDText.com, Inc.

54. Stefan N, Häring HU, Schulze MB. Metabolically healthy obesity: the low-hanging fruit in obesity treatment? The lancet Diabetes & endocrinology. 2018;6(3):249-58.

55. Lindström J, Ilanne-Parikka P, Peltonen M, Aunola S, Eriksson JG, Hemiö K, et al. Sustained reduction in the incidence of type 2 diabetes by lifestyle intervention: follow-up of the Finnish Diabetes Prevention Study. Lancet (London, England). 2006;368(9548):1673-9.

56. Hruby A, Hu FB. The Epidemiology of Obesity: A Big Picture. Pharmacoeconomics. 2015;33(7):673-89.

57. Mandai N, Akazawa K, Hara N, Ide Y, Ide K, Dazai U, et al. Body Weight Reduction Results in Favorable Changes in Blood Pressure, Serum Lipids, and Blood Sugar in Middle-

Aged Japanese Persons: A 5-Year Interval Observational Study of 26,824 Cases. Glob J Health Sci. 2015;7(5):159-70.

58. Kirk S, Brehm B, Saelens BE, Woo JG, Kissel E, D'Alessio D, et al. Role of carbohydrate modification in weight management among obese children: a randomized clinical trial. J Pediatr. 2012;161(2):320-7.e1.

59. Farrokhi F, Smiley D, Umpierrez GE. Glycemic control in non-diabetic critically ill patients. Best practice & research Clinical endocrinology & metabolism. 2011;25(5):813-24.

60. Storey S, Von Ah D, Hammer MJ. Measurement of Hyperglycemia and Impact on Health Outcomes in People With Cancer: Challenges and Opportunities. Oncology nursing forum. 2017;44(4):E141-e51.

61. Collier B, Dossett LA, May AK, Diaz JJ. Glucose control and the inflammatory response. Nutrition in clinical practice : official publication of the American Society for Parenteral and Enteral Nutrition. 2008;23(1):3-15.

62. Brand-Miller J, Hayne S, Petocz P, Colagiuri S. Low-glycemic index diets in the management of diabetes: a meta-analysis of randomized controlled trials. Diabetes care. 2003;26(8):2261-7.

63. Basta G, Schmidt AM, De Caterina R. Advanced glycation end products and vascular inflammation: implications for accelerated atherosclerosis in diabetes. Cardiovascular research. 2004;63(4):582-92.

64. Matheus ASdM, Tannus LRM, Cobas RA, Palma CCS, Negrato CA, Gomes MdB. Impact of Diabetes on Cardiovascular Disease: An Update. International Journal of Hypertension. 2013;2013:653789.

65. Giacco R, Parillo M, Rivellese AA, Lasorella G, Giacco A, D'Episcopo L, et al. Long-term dietary treatment with increased amounts of fiber-rich low-glycemic index natural foods improves blood glucose control and reduces the number of hypoglycemic events in type 1 diabetic patients. Diabetes care. 2000;23(10):1461-6.

66. Peiró C, Romacho T, Azcutia V, Villalobos L, Fernández E, Bolaños JP, et al. Inflammation, glucose, and vascular cell damage: the role of the pentose phosphate pathway. Cardiovascular diabetology. 2016;15:82.

67. Wilcox G. Insulin and insulin resistance. Clin Biochem Rev. 2005;26(2):19-39.

68. Nuttall FQ, Gannon MC. Plasma glucose and insulin response to macronutrients in nondiabetic and NIDDM subjects. Diabetes care. 1991;14(9):824-38.

69. Nathan DM, Davidson MB, DeFronzo RA, Heine RJ, Henry RR, Pratley R, et al. Impaired Fasting Glucose and Impaired Glucose Tolerance. Implications for care. 2007;30(3):753-9.

70. Tuso P. Prediabetes and lifestyle modification: time to prevent a preventable disease. Perm J. 2014;18(3):88-93.

71. Nanchen D, Gencer B, Muller O, Auer R, Aghlmandi S, Heg D, et al. Prognosis of Patients With Familial Hypercholesterolemia After Acute Coronary Syndromes. Circulation. 2016;134(10):698-709.

72. Bonnefont-Rousselot D, Benouda L, Bittar R, Darabi-Amin M, Demondion P, Lesnik P, et al. Antiatherogenic properties of high-density lipoproteins from arterial plasma are attenuated as compared to their counterparts of venous origin. Nutrition, Metabolism and Cardiovascular Diseases. 2020;30(1):33-9.

73. Skretteberg PT, Grundvold I, Kjeldsen SE, Erikssen JE, Sandvik L, Liestøl K, et al. HDL-cholesterol and prediction of coronary heart disease: modified by physical fitness? A 28-year follow-up of apparently healthy men. Atherosclerosis. 2012;220(1):250-6.

74. Hedayatnia M, Asadi Z, Zare-Feyzabadi R, Yaghooti-Khorasani M, Ghazizadeh H, Ghaffarian-Zirak R, et al. Dyslipidemia and cardiovascular disease risk among the MASHAD study population. Lipids in Health and Disease. 2020;19(1):42.

75. Miller M. Dyslipidemia and cardiovascular risk: the importance of early prevention. QJM. 2009;102(9):657-67.

76. Mahalle N, Garg MK, Naik SS, Kulkarni MV. Study of pattern of dyslipidemia and its correlation with cardiovascular risk factors in patients with proven coronary artery disease. Indian J Endocrinol Metab. 2014;18(1):48-55.

77. Rosa CdOB, Dos Santos CA, Leite JIA, Caldas APS, Bressan J. Impact of nutrients and food components on dyslipidemias: what is the evidence? Advances in nutrition (Bethesda, Md). 2015;6(6):703-11.

78. LaRosa JC, Grundy SM, Waters DD, Shear C, Barter P, Fruchart J-C, et al. Intensive Lipid Lowering with Atorvastatin in Patients with Stable Coronary Disease. New England Journal of Medicine. 2005;352(14):1425-35.

79. Cannon CP, Braunwald E, McCabe CH, Rader DJ, Rouleau JL, Belder R, et al. Intensive versus moderate lipid lowering with statins after acute coronary syndromes. The New England journal of medicine. 2004;350(15):1495-504.

80. Chapman MJ. Beyond the statins: new therapeutic perspectives in cardiovascular disease prevention. Cardiovascular drugs and therapy. 2005;19(2):135-9.

81. Egan BM, Kjeldsen SE, Grassi G, Esler M, Mancia G. The global burden of hypertension exceeds 1.4 billion people: should a systolic blood pressure target below 130 become the universal standard? Journal of Hypertension. 2019;37(6):1148-53.

82. Yanai H, Tomono Y, Ito K, Furutani N, Yoshida H, Tada N. The underlying mechanisms for development of hypertension in the metabolic syndrome. Nutr J. 2008;7:10-.

83. Mendizábal Y, Llorens S, Nava E. Hypertension in Metabolic Syndrome: Vascular Pathophysiology. International Journal of Hypertension. 2013;2013:230868.

84. Cheung BMY, Li C. Diabetes and hypertension: is there a common metabolic pathway? Curr Atheroscler Rep. 2012;14(2):160-6.

85. Pietri P, Vyssoulis G, Vlachopoulos C, Zervoudaki A, Gialernios T, Aznaouridis K, et al. Relationship between low-grade inflammation and arterial stiffness in patients with essential hypertension. J Hypertens. 2006;24(11):2231-8.

86. Livingstone KM, McNaughton SA. Diet quality is associated with obesity and hypertension in Australian adults: a cross sectional study. BMC Public Health. 2016;16(1):1037-.

87. Minihane AM, Vinoy S, Russell WR, Baka A, Roche HM, Tuohy KM, et al. Low-grade inflammation, diet composition and health: current research evidence and its translation. The British journal of nutrition. 2015;114(7):999-1012.

88. Edwards T. Inflammation, pain, and chronic disease: an integrative approach to treatment and prevention. Alternative therapies in health and medicine. 2005;11(6):20-7; quiz 8, 75.

89. Robbie L, Libby P. Inflammation and atherothrombosis. Annals of the New York Academy of Sciences. 2001;947:167-79; discussion 79-80.

90. Dandona P, Aljada A, Chaudhuri A, Mohanty P, Garg R. Metabolic syndrome: a comprehensive perspective based on interactions between obesity, diabetes, and inflammation. Circulation. 2005;111(11):1448-54.

91. Pietzner M, Kaul A, Henning AK, Kastenmüller G, Artati A, Lerch MM, et al. Comprehensive metabolic profiling of chronic low-grade inflammation among generally healthy individuals. BMC medicine. 2017;15(1):210.

92. Sproston NR, Ashworth JJ. Role of C-Reactive Protein at Sites of Inflammation and Infection. Front Immunol. 2018;9:754-.

93. Ridker PM. A Test in Context: High-Sensitivity C-Reactive Protein. Journal of the American College of Cardiology. 2016;67(6):712-23.

94. Effoe VS, Correa A, Chen H, Lacy ME, Bertoni AG. High-Sensitivity C-Reactive Protein Is Associated With Incident Type 2 Diabetes Among African Americans: The Jackson Heart Study. Diabetes care. 2015;38(9):1694-700.

95. Nishide R, Ando M, Funabashi H, Yoda Y, Nakano M, Shima M. Association of serum hs-CRP and lipids with obesity in school children in a 12-month follow-up study in Japan. Environ Health Prev Med. 2015;20(2):116-22.

96. Fonseca FA, Izar MC. High-Sensitivity C-Reactive Protein and Cardiovascular Disease Across Countries and Ethnicities. Clinics (Sao Paulo, Brazil). 2016;71(4):235-42.

97. Dinarello CA. Historical insights into cytokines. Eur J Immunol. 2007;37 Suppl 1(Suppl 1):S34-S45.

98. Turner MD, Nedjai B, Hurst T, Pennington DJ. Cytokines and chemokines: At the crossroads of cell signalling and inflammatory disease. Biochimica et Biophysica Acta (BBA) - Molecular Cell Research. 2014;1843(11):2563-82.

99. Akdis M, Burgler S, Crameri R, Eiwegger T, Fujita H, Gomez E, et al. Interleukins, from 1 to 37, and interferon-γ: Receptors, functions, and roles in diseases. Journal of Allergy and Clinical Immunology. 2011;127(3):701-21.e70.

100. Chu W-M. Tumor necrosis factor. Cancer Lett. 2013;328(2):222-5.

101. Aggarwal BB, Shishodia S, Ashikawa K, Bharti AC. The role of TNF and its family members in inflammation and cancer: lessons from gene deletion. Current drug targets Inflammation and allergy. 2002;1(4):327-41.

102. Wajant H, Siegmund D. TNFR1 and TNFR2 in the Control of the Life and Death Balance of Macrophages. Frontiers in Cell and Developmental Biology. 2019;7(91).

103. Brenner D, Blaser H, Mak TW. Regulation of tumour necrosis factor signalling: live or let die. Nature Reviews Immunology. 2015;15(6):362-74.

104. Medler J, Wajant H. Tumor necrosis factor receptor-2 (TNFR2): an overview of an emerging drug target. Expert Opinion on Therapeutic Targets. 2019;23(4):295-307.

105. Rehman K, Akash MSH. Mechanisms of inflammatory responses and development of insulin resistance: how are they interlinked? Journal of Biomedical Science. 2016;23(1):87.

106. de Luca C, Olefsky JM. Inflammation and insulin resistance. FEBS Lett. 2008;582(1):97-105.

107. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. J Clin Invest. 1995;95(5):2409-15.

108. Artwohl M, Brunmair B, Fürnsinn C, Hölzenbein T, Rainer G, Freudenthaler A, et al. Insulin does not regulate glucose transport and metabolism in human endothelium. European Journal of Clinical Investigation. 2007;37(8):643-50.

109. Chroni A, Leondaritis G, Karlsson H. Lipids and Lipoproteins in Atherosclerosis. J Lipids. 2011;2011:160104.

110. van der Kamp JW, Poutanen K, Seal CJ, Richardson DP. The HEALTHGRAIN definition of 'whole grain'. Food Nutr Res. 2014;58:10.3402/fnr.v58.22100.

111. Spaggiari M, Calani L, Folloni S, Ranieri R, Dall'Asta C, Galaverna G. The impact of processing on the phenolic acids, free betaine and choline in Triticum spp. L. whole grains and milling by-products. Food chemistry. 2020;311:125940.

112. Maki KC, Slavin JL, Rains TM, Kris-Etherton PM. Limitations of observational evidence: implications for evidence-based dietary recommendations. Advances in nutrition (Bethesda, Md). 2014;5(1):7-15.

113. Gibson RS, Charrondiere UR, Bell W. Measurement Errors in Dietary Assessment Using Self-Reported 24-Hour Recalls in Low-Income Countries and Strategies for Their Prevention. Advances in Nutrition. 2017;8(6):980-91.

114. Dragsted LO, Gao Q, Praticò G, Manach C, Wishart DS, Scalbert A, et al. Dietary and health biomarkers-time for an update. Genes Nutr. 2017;12:24-.

115. Chen Y, Ross AB, Aman P, Kamal-Eldin A. Alkylresorcinols as markers of whole grain wheat and rye in cereal products. J Agric Food Chem. 2004;52(26):8242-6.

116. Landberg R, Kamal-Eldin A, Andersson A, Vessby B, Aman P. Alkylresorcinols as biomarkers of whole-grain wheat and rye intake: plasma concentration and intake estimated from dietary records. Am J Clin Nutr. 2008;87(4):832-8.

117. Ross AB, Shepherd MJ, Schüpphaus M, Sinclair V, Alfaro B, Kamal-Eldin A, et al. Alkylresorcinols in Cereals and Cereal Products. Journal of Agricultural and Food Chemistry. 2003;51(14):4111-8.

118. Söderholm PP, Koskela AH, Lundin JE, Tikkanen MJ, Adlercreutz HC. Plasma pharmacokinetics of alkylresorcinol metabolites: new candidate biomarkers for whole-grain rye and wheat intake. The American Journal of Clinical Nutrition. 2009;90(5):1167-71.

119. Wierzbicka R, Wu H, Franek M, Kamal-Eldin A, Landberg R. Determination of alkylresorcinols and their metabolites in biological samples by gas chromatography-mass spectrometry. Journal of chromatography B, Analytical technologies in the biomedical and life sciences. 2015;1000:120-9.

120. Landberg R, Marklund M, Kamal-Eldin A, Åman P. An update on alkylresorcinols – Occurrence, bioavailability, bioactivity and utility as biomarkers. Journal of Functional Foods. 2014;7:77-89.

121. Ross AB, Shepherd MJ, Bach Knudsen KE, Glitsø LV, Bowey E, Phillips J, et al. Absorption of dietary alkylresorcinols in ileal-cannulated pigs and rats. The British journal of nutrition. 2003;90(4):787-94.

122. Jansson E, Landberg R, Kamal-Eldin A, Wolk A, Vessby B, Åman P. Presence of alkylresorcinols, potential whole grain biomarkers, in human adipose tissue. British Journal of Nutrition. 2010;104(5):633-6.

123. Wu H, Kolehmainen M, Mykkänen H, Poutanen K, Uusitupa M, Schwab U, et al. Alkylresorcinols in adipose tissue biopsies as biomarkers of whole-grain intake: an exploratory study of responsiveness to advised intake over 12 weeks. European journal of clinical nutrition. 2015;69(11):1244-8.

124. Ziegler JU, Steingass CB, Longin CFH, Würschum T, Carle R, Schweiggert RM. Alkylresorcinol composition allows the differentiation of Triticum spp. having different degrees of ploidy. Journal of Cereal Science. 2015;65:244-51.

125. Sang S, Idehen E, Zhao Y, Chu Y. Emerging science on whole grain intake and inflammation. Nutrition reviews. 2020;78(Supplement_1):21-8.

126. Masters RC, Liese AD, Haffner SM, Wagenknecht LE, Hanley AJ. Whole and refined grain intakes are related to inflammatory protein concentrations in human plasma. The Journal of nutrition. 2010;140(3):587-94.

127. Giacco R, Clemente G, Cipriano D, Luongo D, Viscovo D, Patti L, et al. Effects of the regular consumption of wholemeal wheat foods on cardiovascular risk factors in healthy people. Nutrition, metabolism, and cardiovascular diseases : NMCD. 2010;20(3):186-94.

128. Vitaglione P, Mennella I, Ferracane R, Rivellese AA, Giacco R, Ercolini D, et al. Whole-grain wheat consumption reduces inflammation in a randomized controlled trial on overweight and obese subjects with unhealthy dietary and lifestyle behaviors: role of polyphenols bound to cereal dietary fiber. Am J Clin Nutr. 2015;101(2):251-61.

129. Sandberg JC, Björck IME, Nilsson AC. Effects of whole grain rye, with and without resistant starch type 2 supplementation, on glucose tolerance, gut hormones, inflammation and appetite regulation in an 11-14.5 hour perspective; a randomized controlled study in healthy subjects. Nutr J [Internet]. 2017 2017/04//; 16(1):[25 p.].

130. Roager HM, Vogt JK, Kristensen M, Hansen LBS, Ibrügger S, Mærkedahl RB, et al. Whole grain-rich diet reduces body weight and systemic low-grade inflammation without inducing major changes of the gut microbiome: a randomised cross-over trial. Gut. 2019;68(1):83-93.

131. Kundi ZM, Lee JC-Y, Pihlajamäki J, Chan CB, Leung KS, So SSY, et al. Dietary Fiber from Oat and Rye Brans Ameliorate Western Diet-Induced Body Weight Gain and Hepatic

Inflammation by the Modulation of Short-Chain Fatty Acids, Bile Acids, and Tryptophan Metabolism. Molecular nutrition & food research. 2020:e1900580.

132. Alminger M, Eklund-Jonsson C. Whole-grain cereal products based on a high-fibre barley or oat genotype lower post-prandial glucose and insulin responses in healthy humans. European journal of nutrition. 2008;47(6):294-300.

133. Behall KM, Scholfield DJ, Hallfrisch J. Lipids significantly reduced by diets containing barley in moderately hypercholesterolemic men. Journal of the American College of Nutrition. 2004;23(1):55-62.

134. Martínez I, Lattimer JM, Hubach KL, Case JA, Yang J, Weber CG, et al. Gut microbiome composition is linked to whole grain-induced immunological improvements. ISME J. 2013;7(2):269-80.

135. Zhang X, McGeoch SC, Megson IL, MacRury SM, Johnstone AM, Abraham P, et al. Oat-enriched diet reduces inflammatory status assessed by circulating cell-derived microparticle concentrations in type 2 diabetes. Molecular nutrition & food research. 2014;58(6):1322-32.

136. Pavadhgul P, Bumrungpert A, Harjani Y, Kurilich A. Oat porridge consumption alleviates markers of inflammation and oxidative stress in hypercholesterolemic adults. Asia Pacific journal of clinical nutrition. 2019;28(2):260-5.

137. Suriano F, Neyrinck AM, Verspreet J, Olivares M, Leclercq S, Van de Wiele T, et al. Particle size determines the anti-inflammatory effect of wheat bran in a model of fructose over-consumption: Implication of the gut microbiota. Journal of Functional Foods. 2018;41:155-62.

138. Belobrajdic DP, Lam YY, Mano M, Wittert GA, Bird AR. Cereal based diets modulate some markers of oxidative stress and inflammation in lean and obese Zucker rats. Nutrition & metabolism. 2011;8:27-.

139. Shen T, Wang G, You L, Zhang L, Ren H, Hu W, et al. Polysaccharide from wheat bran induces cytokine expression via the toll-like receptor 4-mediated p38 MAPK signaling pathway and prevents cyclophosphamide-induced immunosuppression in mice. Food Nutr Res. 2017;61(1):1344523-.

140. Liu J, Hao Y, Wang Z, Ni F, Wang Y, Gong L, et al. Identification, Quantification, and Anti-inflammatory Activity of 5-n-Alkylresorcinols from 21 Different Wheat Varieties. Journal of Agricultural and Food Chemistry. 2018;66(35):9241-7.

141. McLoughlin RF, Berthon BS, Jensen ME, Baines KJ, Wood LG. Short-chain fatty acids, prebiotics, synbiotics, and systemic inflammation: a systematic review and meta-analysis. Am J Clin Nutr. 2017;106(3):930-45.

142. Walker AW, Ince J, Duncan SH, Webster LM, Holtrop G, Ze X, et al. Dominant and diet-responsive groups of bacteria within the human colonic microbiota. ISME J. 2011;5(2):220-30.

143. Tap J, Mondot S, Levenez F, Pelletier E, Caron C, Furet JP, et al. Towards the human intestinal microbiota phylogenetic core. Environmental microbiology. 2009;11(10):2574-84.

144. Tremaroli V, Bäckhed F. Functional interactions between the gut microbiota and host metabolism. Nature. 2012;489(7415):242-9.

145. Dabke K, Hendrick G, Devkota S. The gut microbiome and metabolic syndrome. The Journal of Clinical Investigation. 2019;129(10):4050-7.

146. Verkhnyatskaya S, Ferrari M, de Vos P, Walvoort MTC. Shaping the Infant Microbiome With Non-digestible Carbohydrates. Frontiers in microbiology. 2019;10(343).

147. Li J, Hou Q, Zhang J, Xu H, Sun Z, Menghe B, et al. Carbohydrate Staple Food Modulates Gut Microbiota of Mongolians in China. Frontiers in microbiology. 2017;8:484.

148. De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, et al. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proceedings of the National Academy of Sciences. 2010;107(33):14691-6.

149. Dao MC, Everard A, Aron-Wisnewsky J, Sokolovska N, Prifti E, Verger EO, et al. Akkermansia muciniphila and improved metabolic health during a dietary intervention in obesity: relationship with gut microbiome richness and ecology. Gut. 2016;65(3):426-36.

150. Ramirez-Farias C, Slezak K, Fuller Z, Duncan A, Holtrop G, Louis P. Effect of inulin on the human gut microbiota: stimulation of Bifidobacterium adolescentis and Faecalibacterium prausnitzii. British Journal of Nutrition. 2008;101(4):541-50.

151. Pandey KR, Naik SR, Vakil BV. Probiotics, prebiotics and synbiotics- a review. Journal of food science and technology. 2015;52(12):7577-87.

152. Candela M, Perna F, Carnevali P, Vitali B, Ciati R, Gionchetti P, et al. Interaction of probiotic Lactobacillus and Bifidobacterium strains with human intestinal epithelial cells: adhesion properties, competition against enteropathogens and modulation of IL-8 production. International journal of food microbiology. 2008;125(3):286-92.

153. Warmbrunn MV, Herrema H, Aron-Wisnewsky J, Soeters MR, Van Raalte DH, Nieuwdorp M. Gut microbiota: a promising target against cardiometabolic diseases. Expert review of endocrinology & metabolism. 2020;15(1):13-27.

154. Li J-M, Yu R, Zhang L-P, Wen S-Y, Wang S-J, Zhang X-Y, et al. Dietary fructose-induced gut dysbiosis promotes mouse hippocampal neuroinflammation: a benefit of short-chain fatty acids. Microbiome. 2019;7(1):98.

155. Shin N-R, Whon TW, Bae J-W. Proteobacteria: microbial signature of dysbiosis in gut microbiota. Trends in Biotechnology. 2015;33(9):496-503.

156. Gurung M, Li Z, You H, Rodrigues R, Jump DB, Morgun A, et al. Role of gut microbiota in type 2 diabetes pathophysiology. EBioMedicine. 2020;51:102590.

157. Tan J, McKenzie C, Potamitis M, Thorburn AN, Mackay CR, Macia L. The role of short-chain fatty acids in health and disease. Advances in immunology. 2014;121:91-119.

158. den Besten G, van Eunen K, Groen AK, Venema K, Reijngoud DJ, Bakker BM. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. Journal of lipid research. 2013;54(9):2325-40.

159. Puddu A, Sanguineti R, Montecucco F, Viviani GL. Evidence for the Gut Microbiota Short-Chain Fatty Acids as Key Pathophysiological Molecules Improving Diabetes. Mediators of Inflammation. 2014;2014:162021.

160. Topping DL, Clifton PM. Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. Physiological reviews. 2001;81(3):1031-64.

161. Gill PA, van Zelm MC, Muir JG, Gibson PR. Review article: short chain fatty acids as potential therapeutic agents in human gastrointestinal and inflammatory disorders. Alimentary pharmacology & therapeutics. 2018;48(1):15-34.

162. Morrison DJ, Preston T. Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism. Gut Microbes. 2016;7(3):189-200.

163. Chambers ES, Preston T, Frost G, Morrison DJ. Role of Gut Microbiota-Generated Short-Chain Fatty Acids in Metabolic and Cardiovascular Health. Curr Nutr Rep. 2018;7(4):198-206.

164. Gao F, Lv Y-W, Long J, Chen J-M, He J-M, Ruan X-Z, et al. Butyrate Improves the Metabolic Disorder and Gut Microbiome Dysbiosis in Mice Induced by a High-Fat Diet. Front Pharmacol. 2019;10:1040-.

165. Canani RB, Costanzo MD, Leone L, Pedata M, Meli R, Calignano A. Potential beneficial effects of butyrate in intestinal and extraintestinal diseases. World J Gastroenterol. 2011;17(12):1519-28.

166. Vinolo MA, Rodrigues HG, Nachbar RT, Curi R. Regulation of inflammation by short chain fatty acids. Nutrients. 2011;3(10):858-76.

167. Murugesan S, Nirmalkar K, Hoyo-Vadillo C, García-Espitia M, Ramírez-Sánchez D, García-Mena J. Gut microbiome production of short-chain fatty acids and obesity in children. European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology. 2018;37(4):621-5.

168. Roshanravan N, Mahdavi R, Alizadeh E, Jafarabadi MA, Hedayati M, Ghavami A, et al. Effect of Butyrate and Inulin Supplementation on Glycemic Status, Lipid Profile and Glucagon-Like Peptide 1 Level in Patients with Type 2 Diabetes: A Randomized Double-Blind, Placebo-Controlled Trial. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme. 2017;49(11):886-91.

169. Segain JP, Raingeard de la Blétière D, Bourreille A, Leray V, Gervois N, Rosales C, et al. Butyrate inhibits inflammatory responses through NFkappaB inhibition: implications for Crohn's disease. Gut. 2000;47(3):397-403.

170. Valdes AM, Walter J, Segal E, Spector TD. Role of the gut microbiota in nutrition and health. BMJ. 2018;361:k2179.

171. Frost G, Sleeth ML, Sahuri-Arisoylu M, Lizarbe B, Cerdan S, Brody L, et al. The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. Nature Communications. 2014;5(1):3611.

172. De Vadder F, Kovatcheva-Datchary P, Goncalves D, Vinera J, Zitoun C, Duchampt A, et al. Microbiota-generated metabolites promote metabolic benefits via gut-brain neural circuits. Cell. 2014;156(1-2):84-96.

173. Demigné C, Morand C, Levrat M-A, Besson C, Moundras C, Rémésy C. Effect of propionate on fatty acid and cholesterol synthesis and on acetate metabolism in isolated rat hepatocytes. British Journal of Nutrition. 1995;74(2):209-19.

174. Tedelind S, Westberg F, Kjerrulf M, Vidal A. Anti-inflammatory properties of the short-chain fatty acids acetate and propionate: a study with relevance to inflammatory bowel disease. World J Gastroenterol. 2007;13(20):2826-32.

175. Wu W, Sun M, Chen F, Cao AT, Liu H, Zhao Y, et al. Microbiota metabolite short-chain fatty acid acetate promotes intestinal IgA response to microbiota which is mediated by GPR43. Mucosal Immunol. 2017;10(4):946-56.

176. Bray GA. How bad is fructose? The American Journal of Clinical Nutrition. 2007;86(4):895-6.

177. Flynn A. Nutrition and Health | Galactosemia. In: Fuquay JW, editor. Encyclopedia of Dairy Sciences (Second Edition). San Diego: Academic Press; 2011. p. 1051-5.

178. Williams CA. GALACTOSE. In: Caballero B, editor. Encyclopedia of Food Sciences and Nutrition (Second Edition). Oxford: Academic Press; 2003. p. 2843-6.

179. Yao D, Brownlee M. Hyperglycemia-Induced Reactive Oxygen Species Increase Expression of the Receptor for Advanced Glycation End Products (RAGE) and RAGE Ligands. Diabetes. 2010;59(1):249-55.

180. Fakhruddin S, Alanazi W, Jackson KE. Diabetes-Induced Reactive Oxygen Species: Mechanism of Their Generation and Role in Renal Injury. Journal of Diabetes Research. 2017;2017:8379327.

181. Livesey G, Taylor R. Fructose consumption and consequences for glycation, plasma triacylglycerol, and body weight: meta-analyses and meta-regression models of intervention studies. Am J Clin Nutr. 2008;88(5):1419-37.

182. Zhang D-M, Jiao R-Q, Kong L-D. High Dietary Fructose: Direct or Indirect Dangerous Factors Disturbing Tissue and Organ Functions. Nutrients. 2017;9(4):335.

183. Teff KL, Elliott SS, Tschöp M, Kieffer TJ, Rader D, Heiman M, et al. Dietary Fructose Reduces Circulating Insulin and Leptin, Attenuates Postprandial Suppression of Ghrelin, and Increases Triglycerides in Women. The Journal of Clinical Endocrinology & Metabolism. 2004;89(6):2963-72.

184. Havel PJ. Dietary fructose: implications for dysregulation of energy homeostasis and lipid/carbohydrate metabolism. Nutrition reviews. 2005;63(5):133-57.

185. Tappy L. Fructose-containing caloric sweeteners as a cause of obesity and metabolic disorders. The Journal of experimental biology. 2018;221(Pt Suppl 1).

186. Bray GA. Energy and fructose from beverages sweetened with sugar or high-fructose corn syrup pose a health risk for some people. Advances in nutrition (Bethesda, Md). 2013;4(2):220-5.

187. Dekker MJ, Su Q, Baker C, Rutledge AC, Adeli K. Fructose: a highly lipogenic nutrient implicated in insulin resistance, hepatic steatosis, and the metabolic syndrome. American journal of physiology Endocrinology and metabolism. 2010;299(5):E685-94.

188. Stanhope KL, Schwarz JM, Havel PJ. Adverse metabolic effects of dietary fructose: results from the recent epidemiological, clinical, and mechanistic studies. Current opinion in lipidology. 2013;24(3):198-206.

189. Abdulla MH, Sattar MA, Johns EJ. The Relation between Fructose-Induced Metabolic Syndrome and Altered Renal Haemodynamic and Excretory Function in the Rat. Int J Nephrol. 2011;2011:934659-.

190. Pektas MB, Koca HB, Sadi G, Akar F. Dietary Fructose Activates Insulin Signaling and Inflammation in Adipose Tissue: Modulatory Role of Resveratrol. BioMed Research International. 2016;2016:8014252.

191. Kawabata K, Kanmura S, Morinaga Y, Tanaka A, Makino T, Fujita T, et al. A high-fructose diet induces epithelial barrier dysfunction and exacerbates the severity of dextran sulfate sodium-induced colitis. International journal of molecular medicine. 2019;43(3):1487-96.

192. Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, et al. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes. 2007;56(7):1761-72.

193. Coelho AI, Berry GT, Rubio-Gozalbo ME. Galactose metabolism and health. Current Opinion in Clinical Nutrition & Metabolic Care. 2015;18(4).

194. Kowluru RA, Tang J, Kern TS. Abnormalities of retinal metabolism in diabetes and experimental galactosemia. VII. Effect of long-term administration of antioxidants on the development of retinopathy. Diabetes. 2001;50(8):1938-42.

195. Birlouez-Aragon I, Alloussi S. Effect of prolonged galactose consumption on galactose tolerance in young healthy humans. Annals of nutrition & metabolism. 1990;34(1):1-7.

196. Luévano-Contreras C, Gómez-Ojeda A, Macías-Cervantes MH, Garay-Sevilla ME. Dietary Advanced Glycation End Products and Cardiometabolic Risk. Current diabetes reports. 2017;17(8):63.

197. Uribarri J, del Castillo MD, de la Maza MP, Filip R, Gugliucci A, Luevano-Contreras C, et al. Dietary advanced glycation end products and their role in health and disease. Advances in nutrition (Bethesda, Md). 2015;6(4):461-73.

198. Post RE, Mainous AG, 3rd, King DE, Simpson KN. Dietary fiber for the treatment of type 2 diabetes mellitus: a meta-analysis. Journal of the American Board of Family Medicine : JABFM. 2012;25(1):16-23.

199. Hadzi-Petrushev N, Stojkovski V, Mitrov D, Mladenov M. d-galactose induced inflammation lipid peroxidation and platelet activation in rats. Cytokine. 2014;69(1):150-3.

200. Lu J, Zheng YL, Wu DM, Luo L, Sun DX, Shan Q. Ursolic acid ameliorates cognition deficits and attenuates oxidative damage in the brain of senescent mice induced by D-galactose. Biochemical pharmacology. 2007;74(7):1078-90.

201. Bo-Htay C, Palee S, Apaijai N, Chattipakorn SC, Chattipakorn N. Effects of d-galactose-induced ageing on the heart and its potential interventions. Journal of Cellular and Molecular Medicine. 2018;22(3):1392-410.

202. Wallin A, Di Giuseppe D, Burgaz A, Håkansson N, Cederholm T, Michaëlsson K, et al. Validity of food frequency questionnaire-based estimates of long-term long-chain n-3 polyunsaturated fatty acid intake. Eur J Nutr. 2014;53(2):549-55.

203. Landberg R, Andersson S-O, Zhang J-X, Johansson J-E, Stenman U-H, Adlercreutz H, et al. Rye Whole Grain and Bran Intake Compared with Refined Wheat Decreases Urinary C-

Peptide, Plasma Insulin, and Prostate Specific Antigen in Men with Prostate Cancer. The Journal of Nutrition. 2010;140(12):2180-6.

204. Landberg R, Kamal-Eldin A, Andersson S-O, Johansson J-E, Zhang J-X, Hallmans Gr, et al. Reproducibility of Plasma Alkylresorcinols during a 6-Week Rye Intervention Study in Men with Prostate Cancer. The Journal of Nutrition. 2009;139(5):975-80.

205. Axen KV, Axen K. Very low-carbohydrate versus isocaloric high-carbohydrate diet in dietary obese rats. Obesity (Silver Spring, Md). 2006;14(8):1344-52.

206. Chaumontet C, Azzout-Marniche D, Blais A, Chalvon-Dermersay T, Nadkarni NA, Piedcoq J, et al. Rats Prone to Obesity Under a High-Carbohydrate Diet have Increased Post-Meal CCK mRNA Expression and Characteristics of Rats Fed a High-Glycemic Index Diet. Front Nutr. 2015;2:22-.

207. Gamba CA, Friedman SM, Rodriguez PN, Macri EV, Vacas MI, Lifshitz F. Metabolic status in growing rats fed isocaloric diets with increased carbohydrate-to-fat ratio. Nutrition. 2005;21(2):249-54.

208. Zhang Z, He S, Cao X, Ye Y, Yang L, Wang J, et al. Potential prebiotic activities of soybean peptides Maillard reaction products on modulating gut microbiota to alleviate aging-related disorders in D-galactose-induced ICR mice. Journal of Functional Foods. 2020;65:103729.

209. Merino-Aguilar H, Arrieta-Baez D, Jiménez-Estrada M, Magos-Guerrero G, Hernández-Bautista RJ, Susunaga-Notario ADC, et al. Effect of fructooligosaccharides fraction from Psacalium decompositum on inflammation and dyslipidemia in rats with fructose-induced obesity. Nutrients. 2014;6(2):591-604.

210. Capitán-Cañadas F, Ocón B, Aranda CJ, Anzola A, Suárez MD, Zarzuelo A, et al. Fructooligosaccharides exert intestinal anti-inflammatory activity in the CD4+ CD62L+ T cell transfer model of colitis in C57BL/6J mice. European journal of nutrition. 2016;55(4):1445-54.

211. Nor Adila Mhd Omar JF, Johanita Kruger, Federica Dal Bello, Claudio Medana, Massimo Collino, Galia Zamaratskaia, Karl Michaelsson Alicja Wolk, Rikard Landberg. Effects of high intakes of fructose and galactose with or without added fructooligosaccharides on metabolic factors, inflammation and gut integrity in a rat model. Molecular Nutrition & Food Research - Submitted.

212. Sandvik P. Rye bread in Sweden : Health-related and sensory qualities, consumer perceptions and consumption patterns [Doctoral thesis, comprehensive summary]. Uppsala: Acta Universitatis Upsaliensis; 2017.

213. Livsmedels- och näringsintag bland vuxna i Sverige Cited on: 29 October 2020 [Available from: https://www.livsmedelsverket.se/globalassets/publikationsdatabas/rapporter/2011/riksmaten_2010_20111.pdf.

214. Wilkinson RD, Williams R, Scott CJ, Burden RE. Cathepsin S: therapeutic, diagnostic, and prognostic potential. Biological chemistry. 2015;396(8):867-82.

215. Liu J, Ma L, Yang J, Ren A, Sun Z, Yan G, et al. Increased serum cathepsin S in patients with atherosclerosis and diabetes. Atherosclerosis. 2006;186(2):411-9.

216. Naour N, Rouault C, Fellahi S, Lavoie M-E, Poitou C, Keophiphath M, et al. Cathepsins in Human Obesity: Changes in Energy Balance Predominantly Affect Cathepsin S in Adipose Tissue and in Circulation. The Journal of Clinical Endocrinology & Metabolism. 2010;95(4):1861-8.

217. Turk V, Stoka V, Vasiljeva O, Renko M, Sun T, Turk B, et al. Cysteine cathepsins: from structure, function and regulation to new frontiers. Biochimica et biophysica acta. 2012;1824(1):68-88.

218. Lindstedt L, Lee M, Oörni K, Brömme D, Kovanen PT. Cathepsins F and S block HDL3-induced cholesterol efflux from macrophage foam cells. Biochemical and biophysical research communications. 2003;312(4):1019-24.

219. Jobs E, Adamsson V, Larsson A, Jobs M, Nerpin E, Ingelsson E, et al. Influence of a prudent diet on circulating cathepsin S in humans. Nutr J. 2014;13(1):84.

220. O'Reilly MS, Boehm T, Shing Y, Fukai N, Vasios G, Lane WS, et al. Endostatin: An Endogenous Inhibitor of Angiogenesis and Tumor Growth. Cell. 1997;88(2):277-85.

221. Digtyar AV, Pozdnyakova NV, Feldman NB, Lutsenko SV, Severin SE. Endostatin: Current concepts about its biological role and mechanisms of action. Biochemistry (Moscow). 2007;72(3):235-46.

222. Ruge T, Carlsson AC, Larsson A, Ärnlöv J. Endostatin: a promising biomarker in the cardiovascular continuum? Biomarkers in medicine. 2017;11(10):905-16.

223. Carlsson AC, Ruge T, Sundström J, Ingelsson E, Larsson A, Lind L, et al. Association between circulating endostatin, hypertension duration, and hypertensive target-organ damage. Hypertension (Dallas, Tex : 1979). 2013;62(6):1146-51.

224. Nijhawan P, Makkar R, Gupta A, Arora S, Garg M, Behl T. Insights into the role of endostatin in obesity. Obesity Medicine. 2019;15:100120.

225. Panchal VR, Rehman J, Nguyen AT, Brown JW, Turrentine MW, Mahomed Y, et al. Reduced pericardial levels of endostatin correlate with collateral development in patients with ischemic heart disease. Journal of the American College of Cardiology. 2004;43(8):1383-7.

226. Sodha NR, Clements RT, Boodhwani M, Xu S-H, Laham RJ, Bianchi C, et al. Endostatin and angiostatin are increased in diabetic patients with coronary artery disease and associated with impaired coronary collateral formation. Am J Physiol Heart Circ Physiol. 2009;296(2):H428-H34.

227. Wu H, editor Alkylresorcinols in adipose tissue as long-term biomarkers of whole grain wheat and rye Intake2017.

228. Xu Y, Wan Q, Feng J, Du L, Li K, Zhou Y. Whole grain diet reduces systemic inflammation: A meta-analysis of 9 randomized trials. Medicine (Baltimore). 2018;97(43):e12995-e.

229. Kallio P, Kolehmainen M, Laaksonen DE, Pulkkinen L, Atalay M, Mykkänen H, et al. Inflammation markers are modulated by responses to diets differing in postprandial insulin responses in individuals with the metabolic syndrome. Am J Clin Nutr. 2008;87(5):1497-503.

230. Nordström T, Akre O, Aly M, Grönberg H, Eklund M. Prostate-specific antigen (PSA) density in the diagnostic algorithm of prostate cancer. Prostate cancer and prostatic diseases. 2018;21(1):57-63.

231. Harjunpää H, Llort Asens M, Guenther C, Fagerholm SC. Cell Adhesion Molecules and Their Roles and Regulation in the Immune and Tumor Microenvironment. Front Immunol. 2019;10:1078-.

232. Ren Z, Kang W, Wang L, Sun B, Ma J, Zheng C, et al. E2F1 renders prostate cancer cell resistant to ICAM-1 mediated antitumor immunity by NF-κB modulation. Molecular Cancer. 2014;13(1):84.

233. Nishida N, Yano H, Nishida T, Kamura T, Kojiro M. Angiogenesis in cancer. Vasc Health Risk Manag. 2006;2(3):213-9.

234. Ricote M, García-Tuñón I, Bethencourt FR, Fraile B, Paniagua R, Royuela M. Interleukin-1 (IL-1alpha and IL-1beta) and its receptors (IL-1RI, IL-1RII, and IL-1Ra) in prostate carcinoma. Cancer. 2004;100(7):1388-96.

235. Royuela M, Rodríguez-Berriguete G, Fraile B, Paniagua R. TNF-alpha/IL-1/NF-kappaB transduction pathway in human cancer prostate. Histology and histopathology. 2008;23(10):1279-90.

236. Voronov E, Carmi Y, Apte RN. The role IL-1 in tumor-mediated angiogenesis. Front Physiol. 2014;5:114.

237. Bocarsly ME, Powell ES, Avena NM, Hoebel BG. High-fructose corn syrup causes characteristics of obesity in rats: increased body weight, body fat and triglyceride levels. Pharmacol Biochem Behav. 2010;97(1):101-6.

238. OZCAN SINIR G, SUNA S, INAN S, BAGDAS D, TAMER CE, COPUR OU, et al. Effects of long-term consumption of high fructose corn syrup containing peach nectar on body weight gain in sprague dawley rats. Food Science and Technology. 2017;37:337-43.

239. Ramos VW, Batista LO, Albuquerque KT. Effects of fructose consumption on food intake and biochemical and body parameters in Wistar rats. Revista Portuguesa de Cardiologia (English Edition). 2017;36(12):937-41.

240. Ji L, Li C, Shen N, Huan Y, Liu Q, Liu S, et al. A simple and stable galactosemic cataract model for rats. Int J Clin Exp Med. 2015;8(8):12874-81.

241. Bank N, Coco M, Aynedjian HS. Galactose feeding causes glomerular hyperperfusion: prevention by aldose reductase inhibition. The American journal of physiology. 1989;256(6 Pt 2):F994-9.

242. Patterson JW. Hyperglycemia and Galactose Cataracts. American Journal of Physiology-Legacy Content. 1954;177(3):541-3.

243. Barosa C, Silva C, Fagulha A, Barros L, Caldeira MM, Carvalheiro M, et al. Sources of hepatic glycogen synthesis following a milk-containing breakfast meal in healthy subjects. Metabolism. 2012;61(2):250-4.

244. Ramana BV, Kumar VV, Krishna PN, Kumar CS, Reddy PU, Raju TN. Effect of quercetin on galactose-induced hyperglycaemic oxidative stress in hepatic and neuronal tissues of Wistar rats. Acta diabetologica. 2006;43(4):135-41.

245. Stahel P, Kim JJ, Xiao C, Cant JP. Of the milk sugars, galactose, but not prebiotic galacto-oligosaccharide, improves insulin sensitivity in male Sprague-Dawley rats. PLoS One. 2017;12(2):e0172260.

246. Clemens RA, Jones JM, Kern M, Lee S-Y, Mayhew EJ, Slavin JL, et al. Functionality of Sugars in Foods and Health. Comprehensive Reviews in Food Science and Food Safety. 2016;15(3):433-70.

247. Ercan N, Nuttall FQ, Gannon MC, Redmon JB, Sheridan KJ. Effects of glucose, galactose, and lactose ingestion on the plasma glucose and insulin response in persons with non-insulin-dependent diabetes mellitus. Metabolism. 1993;42(12):1560-7.

248. Knezovic A, Osmanovic Barilar J, Babic A, Bagaric R, Farkas V, Riederer P, et al. Glucagon-like peptide-1 mediates effects of oral galactose in streptozotocin-induced rat model of sporadic Alzheimer's disease. Neuropharmacology. 2018;135:48-62.

249. Tolhurst G, Heffron H, Lam YS, Parker HE, Habib AM, Diakogiannaki E, et al. Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein-coupled receptor FFAR2. Diabetes. 2012;61(2):364-71.

250. DiNicolantonio JJ, Lucan SC, O'Keefe JH. The Evidence for Saturated Fat and for Sugar Related to Coronary Heart Disease. Prog Cardiovasc Dis. 2016;58(5):464-72.

251. Jameel F, Phang M, Wood LG, Garg ML. Acute effects of feeding fructose, glucose and sucrose on blood lipid levels and systemic inflammation. Lipids in Health and Disease. 2014;13(1):195.

252. Zhu SY, Jiang N, Tu J, Yang J, Zhou Y. Antioxidant and Anti-aging Activities of Silybum Marianum Protein Hydrolysate in Mice Treated with D-galactose. Biomedical and environmental sciences : BES. 2017;30(9):623-31.

253. Lusis AJ. Atherosclerosis. Nature. 2000;407(6801):233-41.

254. Expert Panel on Detection E, Adults ToHBCi. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA. 2001;285(19):2486-97.

255. Virtanen JK, Mursu J, Voutilainen S, Tuomainen TP. The associations of serum n-6 polyunsaturated fatty acids with serum C-reactive protein in men: the Kuopio Ischaemic Heart Disease Risk Factor Study. European journal of clinical nutrition. 2018;72(3):342-8.

256. Halfen S, Jacometo CB, Mattei P, Fenstenseifer SR, Pfeifer LFM, Pino FABD, et al. Diets Rich in Polyunsaturated Fatty Acids With Different Omega-6/Omega-3 Ratio Decrease Liver Content of Saturated Fatty Acids Across Generations of Wistar Rats. Brazilian Archives of Biology and Technology. 2016;59.

257. Aragno M, Mastrocola R. Dietary Sugars and Endogenous Formation of Advanced Glycation Endproducts: Emerging Mechanisms of Disease. Nutrients. 2017;9(4):385.

258. Song X, Bao M, Li D, Li YM. Advanced glycation in D-galactose induced mouse aging model. Mech Ageing Dev. 1999;108(3):239-51.

259. Suliman ME, Heimbürger O, Bárány P, Anderstam B, Pecoits-Filho R, Rodríguez Ayala E, et al. Plasma pentosidine is associated with inflammation and malnutrition in end-stage renal

disease patients starting on dialysis therapy. Journal of the American Society of Nephrology : JASN. 2003;14(6):1614-22.

260. Ansari NA, Moinuddin, Ali R. Glycated Lysine Residues: A Marker for Non-Enzymatic Protein Glycation in Age-Related Diseases. Disease Markers. 2011;30:718694.

261. Awasthi S, Saraswathi NT. Non-enzymatic glycation mediated structure–function changes in proteins: case of serum albumin. RSC Advances. 2016;6(93):90739-53.

262. Assimakopoulos SF, Triantos C, Maroulis I, Gogos C. The Role of the Gut Barrier Function in Health and Disease. Gastroenterology Res. 2018;11(4):261-3.

263. Amar J, Chabo C, Waget A, Klopp P, Vachoux C, Bermúdez-Humarán LG, et al. Intestinal mucosal adherence and translocation of commensal bacteria at the early onset of type 2 diabetes: molecular mechanisms and probiotic treatment. EMBO molecular medicine. 2011;3(9):559-72.

264. Fasano A. Zonulin, regulation of tight junctions, and autoimmune diseases. Annals of the New York Academy of Sciences. 2012;1258(1):25-33.

265. Louis P, Flint HJ. Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. FEMS Microbiology Letters. 2009;294(1):1-8.

266. Liu F, Li P, Chen M, Luo Y, Prabhakar M, Zheng H, et al. Fructooligosaccharide (FOS) and Galactooligosaccharide (GOS) Increase Bifidobacterium but Reduce Butyrate Producing Bacteria with Adverse Glycemic Metabolism in healthy young population. Scientific reports. 2017;7(1):11789-.

267. Ebert K, Witt H. Fructose malabsorption. Molecular and Cellular Pediatrics. 2016;3(1):10.

268. Kyaw MH, Mayberry JF. Fructose Malabsorption: True Condition or a Variance From Normality. Journal of Clinical Gastroenterology. 2011;45(1):16-21.

269. Le TK, Hosaka T, Nguyen TT, Kassu A, Dang TO, Tran HB, et al. Bifidobacterium species lower serum glucose, increase expressions of insulin signaling proteins, and improve adipokine profile in diabetic mice. Biomedical research (Tokyo, Japan). 2015;36(1):63-70.

270. Watzl B, Girrbach S, Roller M. Inulin, oligofructose and immunomodulation. The British journal of nutrition. 2005;93 Suppl 1:S49-55.

271. Vojinovic D, Radjabzadeh D, Kurilshikov A, Amin N, Wijmenga C, Franke L, et al. Relationship between gut microbiota and circulating metabolites in population-based cohorts. Nature Communications. 2019;10(1):5813.

272. Marchesi JR, Adams DH, Fava F, Hermes GDA, Hirschfield GM, Hold G, et al. The gut microbiota and host health: a new clinical frontier. Gut. 2016;65(2):330-9.

273. Park JY, Mitrou PN, Keogh RH, Luben RN, Wareham NJ, Khaw KT. Effects of body size and sociodemographic characteristics on differences between self-reported and measured anthropometric data in middle-aged men and women: the EPIC-Norfolk study. European journal of clinical nutrition. 2011;65(3):357-67.

274. Pourhoseingholi MA, Baghestani AR, Vahedi M. How to control confounding effects by statistical analysis. Gastroenterol Hepatol Bed Bench. 2012;5(2):79-83.

275. Hackshaw A. Small studies: strengths and limitations. The European respiratory journal. 2008;32(5):1141-3.

276. Nguyen TLA, Vieira-Silva S, Liston A, Raes J. How informative is the mouse for human gut microbiota research? Disease Models & Mechanisms. 2015;8(1):1-16.

277. Williams PG. Evaluation of the evidence between consumption of refined grains and health outcomes. Nutrition reviews. 2012;70(2):80-99.

278. Laitinen K, Mokkala K. Overall Dietary Quality Relates to Gut Microbiota Diversity and Abundance. Int J Mol Sci. 2019;20(8):1835.

279. Qi L, van Dam RM, Liu S, Franz M, Mantzoros C, Hu FB. Whole-grain, bran, and cereal fiber intakes and markers of systemic inflammation in diabetic women. Diabetes care. 2006;29(2):207-11.

280. Wolever TM, Gibbs AL, Mehling C, Chiasson JL, Connelly PW, Josse RG, et al. The Canadian Trial of Carbohydrates in Diabetes (CCD), a 1-y controlled trial of low-glycemic-index dietary carbohydrate in type 2 diabetes: no effect on glycated hemoglobin but reduction in C-reactive protein. Am J Clin Nutr. 2008;87(1):114-25.

281. Ross AB, Pineau N, Kochhar S, Bourgeois A, Beaumont M, Decarli B. Validation of a FFQ for estimating whole-grain cereal food intake. The British journal of nutrition. 2009;102(11):1547-51.

282. Hooper LV, Wong MH, Thelin A, Hansson L, Falk PG, Gordon JI. Molecular analysis of commensal host-microbial relationships in the intestine. Science (New York, NY). 2001;291(5505):881-4.

283. Lallès J-P. Recent advances in intestinal alkaline phosphatase, inflammation, and nutrition. Nutrition reviews. 2019;77(10):710-24.